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Study of semen parameters in captive brown bears (Ursus arctos)

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Abstract
As the Carpathian brown bear still represents one of the biggest populations of bears across Europe, the study of its semen parameters as well as cryopreservation can help advancing the research regarding conservation of genetic diversity for the endangered sub-species of European brown bear. The aim of this paper was to study the parameters of the Carpathian brown bear semen and to attempt its liquid storage at 4°C. The study was performed on two bears, one belonging to the Turda zoo and another to the Libearty bear sanctuary in Zarnesti. We obtained one sample of semen by electro-ejaculation from an 18 years old captive brown bear from the Turda zoo, while the Libearty bear sanctuary provided us with a pair of testicles from a 2.2 years old male, from which we obtained semen by retrograde flushing of the epididymis. We analyzed those samples in 2 different extenders, before and after 24h refrigeration, using the CASA system for concentration and motility and the flow-cytometer for viability. The results showed that semen obtained by electro-ejaculation had good motility, which decreased after 24h of refrigeration, while viability was adequately maintained. The epididymal extraction also provided semen with high motility and viability, but liquid storage at 4°C was unsuccessful. This study is just a beginning, as it needs to be further continued, in order to reach the goal of creating a sperm bank for the Carpathian brown bear and offer new data that could help the preservation of endangered populations of other European brown bears.
Key words: *brown bear, semen collection, CASA, flow cytometry*

Introduction

Bears are a large-bodied mammal of the order Carnivora of the family Ursidae. There are 8 species of bears around the world, living in 4 continents, in a variety of habitats from the tropical rainforest, to the arctic ice. The present research is focused on the brown bear (Ursus arctos), more specifically the Eurasian sub-specie (Ursus arctos arctos), which is one of the largest mammals of Europe, together with the lynx, the wolf and the wolverine (2). Studies showed that brown bears were an essential part of the nitrogen cycle in their ecosystem, spreading nitrogen around with salmon's carcass they had eaten, or by their urination and defecation in the environment (6). Moreover, studies by Gittleman in 2001 (4) insist on showing the consequence of predator's disappearance on the prey population. The non-regulated increase of prey populations has a direct impact on the balance of flora, which then lead to an ill equilibrium of the ecosystem. Romania still has one of the largest brown bear population of Europe, with approximately 7,000 individuals in it. The Romanian bear population even outnumbers the maximal capacity of their habitat and causes problems with the people surrounding it (3). When the drastic diminution in bear populations amongst others all over Europe was noticed in the 1980s, the European commission decided to put in place a juridical tool with the goal to protect the wild fauna and flora of Europe as well as its natural habitats. At the level of the bear populations, they decided to track them, study them in order to prevent future needs. They also decided to translocate bears of the same clade from northern region of Europe to add females and allow the population to grow faster, as a single male is capable of mating with multiple females (7,8). The establishment of a specific genetic resource bank requires the development of sperm cryopreservation procedures in brown bears (1,6). The sperm cryopreservation development is complicated by many issues like the lack of specific studies on the subject and problems on the quality of sperm during harvesting. The large

population of brown bears located in Romania could be used as a surrogate for the European brown bear, in order to study the sperm specific parameters and find protocols for successful cryopreservation and insemination. The aim of this study was to learn more about the Romanian brown bear semen parameters and the effect of two extenders, Triladyl and TRIS-based, on the quality of samples, before and after liquid storage at 4°C.

Materials and methods

The first semen sample was collected on a captive brown bear from the Turda Zoo, when it was transferred to Târgu-Mureș Zoo. The male was eighteen years old and weighed around 350 kilograms. The whole protocol included general anesthesia, monitoring and electro-ejaculation.

The anesthetic protocol: the bear was anesthetized with a cocktail of Zoletil (Tiletamine-Zolazepam) and Xylazine. It received four vials of Zoletil 50, each vial containing 5 ml with 25mg/ml of tiletamine and 25mg/ml of zolazepam, so a total of 500 mg of tiletamine and 500 mg of zolazepam. It also received 16.5 ml of 2% xylazine, so a total of 330 mg of xylazine. The total dose was divided in four separate anesthetic darts which were shot on four different spots, while the bear was still in its isolation cage. Although the bear managed to remove some of them after they were shot, the fluid had already been discharged and the general anesthesia settled very quickly, in 2-3 minutes. The male was then removed from his cage and pulled next to the transportation wagon that was already prepared. The vitals were checked, and all parameters were within normal limits (pulse, respiration rate), while the animal was completely numb, in lateral recumbency. Ophthalmic ointment was applied on the cornea so it would not become dry as the eyes were opened, and the bear could not blink.

For the electro-ejaculation protocol: the hair around the preputial opening was clipped and the area was cleaned with dry paper towel. The probe of the electro-stimulator (Premier 1) was inserted into the rectum and stimuli were gradually applied. Erection was initially very weak, and several electric stimuli were applied until a slight erection was obtained. The penis was manually manipulated in order to remove it out of the prepuce. Initially a small amount of watery liquid was obtained and in order to avoid urine contamination, the collection bottle was changed multiple times. After several more stimuli, a semen sample was obtained. The sample's motility and density were subjectively evaluated under the microscope and then diluted.

One ml of the semen sample was diluted using a TRIS-based extender. Another ml was diluted with the commercial extender Triladyl, while a third ml was left raw. The dilution in all those tubes was performed in a 1:1 ratio with the extenders at 37°C, allowed to cool to environmental temperature, which was around 27°C and then another ml of extender was added. The diluted samples were transported to the laboratory within 45 min, where they were analyzed using the CASA system and flow-cytometry and then submitted to refrigeration for 24 hours.

The second semen sample was acquired from the testicles of a 2.2 years old male, thanks to a collaboration with the Libearty Bear Sanctuary, where they castrate their male bears in order to avoid reproduction.

Anesthesia was induced with a detomidine and ketamine cocktail, using a hypodermic gun. As soon as the male was asleep, the surgical procedure took around ten minutes, and then anesthesia was reversed with Atipamezole. After castration, the testicles were kept on ice for 24h before they could be processed in the laboratory, due to transportation and organizational issues.

The protocol of semen collection from the testicles involved their dissection in order to retrieve the epididymis. For each testicle, the connective tissue and the tunica vaginalis was removed and an epididymo-deferentectomy was performed in order to isolate the cauda epididymis and vas deferens from the testicle. The standard technique was used, consisting of catheterizing the

vas deferens with an 18-gauge needle and retrograde flushing of the vas deferens and epididymis with 5 ml of warmed (37°C) commercial Triladyl extender for one testicle and TRIS-based extender for the other testicle.

Preparation of the sample for CASA was done by diluting 1µl of raw semen with the respective extender in a 1:300 ratio. It was then placed on a prewarmed Leja slide, while analysis was performed using the appropriate CASA modules.

Sperm viability was assessed using a BD FACS Canto II flow. Staining was performed using SYBR-14/PI according to the following protocol: 10µl sperm was diluted at 1:100 ratio and 4µl SYBR-14 as well as 1µl PI were added. The three components were mixed and incubated for 20 minutes at 37°C. Examination was performed right after the incubation. Fluorescence compensation was performed, as SYBR-14 and PI spectra slightly overlap. Detection of fluorescence was made using the 488nm, blue, air-cooled, 20 mW solid state excitation laser, as well as the 530/30 filter for SYBR-14 and 575/26 filter for PI. FACSDiva 6.1.2 was the software used for analysis.

Results

The volume of the sample obtained by electroejaculation was about 3 ml, and had a creamy white color, without any abnormal smell. A droplet was put under the microscope for a subjective evaluation of motility, which was about 80%, and density, which was rare (below 0.5 billion/ml).

The results obtained for CASA evaluation of fresh bear semen obtained by electroejaculation and diluted with TRIS-based and Triladyl extenders are shown in table 1.

Table 1 CASA evaluation fresh bear semen obtained by electroejaculation, diluted with TRIS-based and Triladyl extenders

	Concentration (million per ml)	**Concentration (million per sample)**	**Motility (%)**	**Motile spz. (million per ml)**	**Motile spz. (million per sample)**	**Progressive motility (%)**
Sample 1 TRIS	28,40	85,20	82,42	23,41	70,42	36,91
Sample 1 Triladyl	28,61	85,83	81,27	23,25	69,75	31,15

Results of for CASA motility after 24 h of refrigeration of bear semen obtained by electroejaculation diluted with TRIS-based and Triladyl extenders are shown in table 2:

Table 2 CASA motility after 24 h of refrigeration of bear semen obtained by electroejaculation, diluted with TRIS-based and Triladyl extenders

	Motility (%)	**Progressive motility (%)**
Sample 1 TRIS 24 h	77,87	21,15
Sample 1 Triladyl 24 h	71,09	17,47

Results obtained for sperm viability by flow cytometry on fresh bear semen obtained by electroejaculation, diluted with TRIS-based and Triladyl extenders are shown in table 3:

Table 3 Sperm viability of fresh bear semen obtained by electroejaculation

	Triladyl	TRIS
Viability (%)	92,0	99,7

Results obtained for sperm viability by flow cytometry on bear semen obtained by electroejaculation, after 24h of refrigeration in TRIS-based and Triladyl extenders, are shown in table 4:

Table 4 Sperm viability of fresh bear semen obtained by electroejaculation

	Triladyl	TRIS
Viability (%)	91,2	99,1

For the second sample, flushing of semen from the epididymis was successful for both testicles, while the microscopic examination showed a lot of immature spermatozoa, which is normal, as the bear was only 2.2 years old. Nevertheless, subjective motility was 85%.

The results obtained for CASA evaluation of fresh bear semen obtained by retrograde flushing of the epididymis and diluted with TRIS-based and Triladyl extenders are shown in table 5. After 24h of refrigeration, the subjective analysis showed that all spermatozoa had died.

Table 5 CASA evaluation fresh bear semen obtained by retrograde flushing of the epididymis, diluted with the TRIS-based and Triladyl extenders

	Concentration (million per ml)	Concentration (million per sample)	Motility (%)	Motile spz. (million per ml)	Motile spz. (million per sample)	Progressive motility (%)
Sample 2 TRIS	11,85	8,30	84,65	10,03	7,02	79,61
Sample 2 Triladyl	42,0	42,0	89,19	37,46	37,46	75,14

The results of viability for sample 2 using flow-cytometry was above 99% for both extenders, with slightly better results for the TRIS-based extender (table 6).

Table 6 Sperm viability of fresh bear semen obtained by retrograde flushing of the epididymis

	Triladyl	TRIS
Viability (%)	99.2	99.9

This study is just a timid beginning of what we desire it to be, an ampler research on the conservation of genetic material in brown bear. We did not have enough samples for conclusive results, but we still considered them worth presenting, as they represent the first such attempts in Romania and we hope to raise awareness and open new opportunities for further research and collaboration.

The collaboration with the bear sanctuary Libearty is a real opportunity to study the semen from epididymal extraction, which is understudied worldwide in brown bears, and real source of

genetic diversity for conservation, once the ideal protocol for cryopreservation of brown bear semen is found. This ampler research could help find the ideal protocol for the other sub-species of brown bears endangered, as the Romanian brown bear could be studied as a surrogate. The biological material collected with the collaboration of the Libearty bear sanctuary could become a bank of great genetic diversity and resolve present and future inbreeding problems in zoos and in the wild, as it could give more time for long term solutions, like management of corridors against fragmentation of the habitat, resolution of conflicts, management of anti-poacher strategies, etc.

Moreover, those results add elements to the understanding of the sperm cells in this species and help to further push the studies in order to find the best protocol for the creation of a sperm bank.

The results obtained for the sample 1 make us confident that in the future, we will be able to establish a stable protocol for bear semen refrigeration, and allow us to hope that bear semen freezing will be possible with a high success rate, as it is already possible and done but with a low success rate. Therefore, I will allow artificial insemination, for a greater genetic diversity as resolution of inbreeding problems in bear populations.

The fact that sample 2 did not survive the 24h refrigeration shows that the male needs to be sexually mature for semen conservation, as most of the sample 2 spermatozoa were immature, with the droplet still present in the middle of their tail.

Although, the motility decreases after 24h refrigeration, the viability is adequate, so semen with a low motility but high viability could be used for in vitro fertilization by ICSI, as the head of the spermatozoon is still in good condition, with intact genetic information, but not being able to move, and therefore unable to normally fertilize an oocyte.

Conclusions

The protocol for tele-anesthesia involving tiletamine/zolazepam + xylazine proved to be extremely efficient, as all maneuvers could be performed safely, and the recovery of the bear thereafter was without any negative incidents.

The main drawback of semen collection using an electro-stimulator is the possible urine contamination of the sample, but this can be avoided by carefully observing the viscosity of the ejaculate and frequently changing the collection bottles.

During general anesthesia for semen collection, various other procedures can be performed, like general and specific examination of the animal, blood sampling, treating various other conditions (like wounds, infections, etc.).

Semen collected by electroejaculation proved to have adequate parameters, with a slightly decreased progressive motility. Motility decreased after 24h of refrigeration, but coupled with the viability results, the semen can still be used for in vitro fertilization by ICSI.

Semen collection by retrograde flushing from the epididymis proved to be an efficient technique, with great potential regarding sperm collection from bears after castration.

Taking into consideration the young age of the bear and the 24h that passed from castration until the testicles were processed, we consider the results obtained for sample 2 as being very positive, showing that technique can be further used for sexually mature bears. CASA and flow-cytometry are powerful tools of sperm analysis and are mandatory for such biodiversity conservation studies, as they provide accurate and objective results.

Our research needs to be continued in order to achieve the goal of repopulating the areas where the brown bear used to live in the past and where it became extinct.

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"Incidence of some intoxications evolution in Romania in *Apis mellifera carpathica* bees monitored in a bee disease prevention program in the active beekeeping season of 2019"

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Abstract
The aim of this paper consisted in evaluating the intoxication cases and their dynamics during the active beekeeping season of 2019 for Apis mellifera carpathica bees monitored in a program for the prevention of infectious and non-infectious diseases. Following the corroboration of the anamnestic data with the morphoclinical data, suspicion of intoxication with toxic feed (pollen), chemicals (pesticides) and medicinal products (antiparasitic products) was established, excluding other causes of illness. During the period of the study, 113 apiaries from different geographic areas of Romania were monitored; counting a number of 7007 bee families, and was identified a number of 18 apiaries (16%) with susceptibility of intoxication, including a number of 1582 bee families (22.57%). The percentage dynamics of the intoxication cases in the studied bee families was the following: 34.07% intoxication with toxic food, 59.6% intoxication with chemical substances and 6.33% intoxication with drugs. We mention that this proportion of the intoxication was on the background of an active beekeeping season in 2019 with many rainfall and extreme weather phenomena.
Key words*: bees, Apis mellifera carpathica, suspicions of intoxication*

Introduction

Pollinators are a key component of the global biodiversity, providing vital ecosystem services to cultivated and wild plants. There are clear evidence regarding the massive decline of both wild and domesticated pollinators (honey bees), but also the parallel decline of the plants that rely on them. Lowering the pollinators number may lead to the loss of pollination services, with considerable ecological and economical negative impacts that could significantly affect the maintenance of wildlife diversity, the stability of larger ecosystems, plant production, food security and human well-being (Potts G. Simon et al ., 2010).

Bees' intoxications are pathological conditions caused by certain organic or inorganic substances which by direct contact or ingestion cause serious disturbances of cellular metabolism and endanger their vital functions. From the etiological point of view, bees' intoxication is classified as: toxic food intoxication (pollen, honey), chemical substances intoxication (pesticides, paints, artificial combs) and drug intoxication (antiparasitic veterinary products).

Drugs intoxication in bees is quite common, causing behavioural changes (abandonment of the hive, stretched wings, agglomeration of bees on the beehive wall, fall of bees in the grass in front of the hive, trembling of the abdomen) and their death on the bottom of the hive (1, 2, 5, 7).

Intoxication with allelochemicals from the harvesting plants used by bees (alkaloids, coumarins, saponins, cyanogenic and cardiac glycosides, and terpenes) has an important share in bees' morbidity, causing depopulation and economic losses for the beekeepers (3, 12).

Chemical toxicosis is the most dangerous non-contagious bee disease, being produced by phytopharmaceuticals used in agricultural and forestry plant protection (12). The most important of the chemical intoxications is the pesticide intoxication, which affects on one hand the digestive

system (as a result of the ingestion of pesticides with food) and, on the other hand, the bee's nervous and respiratory system, together with the environmental pollution, with effects on all the creatures on our planet (4, 6, 8, 10, 11, 12, 13, 14, 15). Pesticides cause a multitude of sublethal effects on bees, affecting their productive performance, the development of the juvenile, lesioning the nervous system (impairment of learning, mobility and memory), increasing the susceptibility to diseases and affecting the hygiene behaviour in the hive (8).

Recent research has shown the interaction between pesticides, especially neonicotinoid pesticides and pathogenic bee viruses, fact that can lead to significant losses in the bee families (4), but also to the worsening of *Nosema ceranae* infections, which together lead to the collapse of the bees colony (8, 13).

Compared with other insects, bees are highly susceptible to pesticides due to deficiencies in genetically encoded detoxification enzymes (15).

Materials and methods

During the study period, the active beehive season of 2019 (February-July), 113 apiaries from the North, Central, East, South and West of the country were monitored, totalling a number of 7007 bee families.

Laboratory tests for the diagnosis of bees' diseases were carried out in the *Beekeeping* Research and Development Institute (ICDA) Laboratory, and the methodology of the laboratory investigations for the diagnosis of bees' diseases was carried out in accordance with the O.I.E. protocols (*World Organization for Animal Health*, 2008).

Results and discussion

Performing a detailed anamnesis, corroborated with the morphoclinical exam of the bee samples (alive, death), completed with the symptomatological picture (conducted by the veterinarians of the ICDA Beekeeping Pathology Laboratory, helped by the beekeepers from the field) after eliminating other causes (infectious, parasitic or technological) followed by laboratory examinations (direct microscopy of the intestinal content, bacterioscopic, bacteriological, mycological and parasitological exams) the suspicion of intoxication in the studied bees was established.

From a total number of 113 monitored apiaries, summing 7007 bee families, 18 apiaries had intoxication problems (15.92%), representing a total of 1582 bee families (22.57%) (Table 1).

Apiaries with suspicion of intoxication throughout the entire period of the study (active beekeeping year 2019)

Table 1

Active beekeeping year	**2019**
Number of monitored apiaries	113
Number of bee families monitored	7007
Number of apiaries affected by intoxication	18 (15.92 %)
Number of bee families suspected of intoxication	1582 (22.57%)

From a number of 7007 monitored bee families, 1582 bee families (22.57%) were identified with the suspicion of intoxication, of which mortality was found in 30 bee families

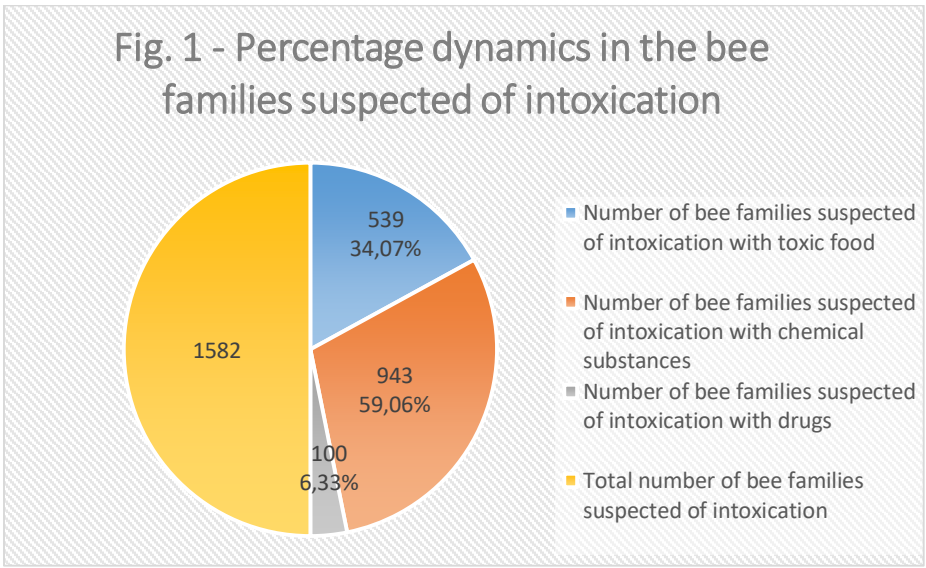
(1.88%), the rest showing depopulation. The percentage dynamics of intoxication cases in the studied bee families was the following: 34.07% toxic food intoxication, 59.6% chemical substances intoxication and 6.33% drugs intoxication (Table 2). We mention that this proportion of intoxication was on the background of an active beekeeping season 2019 with abundant rain and rainwater puddles.

Types of suspected intoxication in the bee families monitored during the period of the active season 2019

Table 2

Type of intoxication	Number of bee families with intoxication suspicion/ active season of 2019	Number of dead bees/ active season of 2019
Number of bee families intoxicated with *toxic food (pollen)*	539 (34.07%)	30 (1.88%)
Number of bee families intoxicated with *chemical substances*	943 (59.6 %)	0
Number of bee families intoxicated with *drugs*	100 (6.33 %)	0
Total number of intoxicated bee families	1582	30 (1.88%)

From Table 2 it is found that during the active season of 2019, the intoxications with sublethal doses of toxic chemical substances had the largest share, being found in 943 bee families (59.6%), followed by the intoxications with toxic food in 539 bee families (34.07%) and drugs intoxications in 100 bee families (6.33%) (Fig.1).



The explanation for this percentage, with the predominance of intoxications with toxic chemical substances, can be attributed to the fact that the beekeeping season of 2019 was a rainy season that allowed rainwater to form puddles and to increase therefore its concentration in chemicals that are toxic to bee. These toxic substances originated either from spraying the

agricultural crops with various pesticides, or by treating the seeds from different crops with toxic chemicals, substances that were emitted into the soil and then concentrated in puddles' water, or from treatments planned by the town halls in order to combat mosquitoes. The low proportion of drug intoxications is explained by the fact that during the main harvesting periods no hive deworming treatments are being carried out and as such, this type of intrusions accidentally occur, either at the beginning of the active season or between large harvesting periods. The distribution of the intoxication suspected cases by counties and months is presented in Table 3.

Distribution of the cases with intoxication suspicion between January and July 2019

Table 3

Month /2019	**Number of affected apiaries**	**County**	**CLINICAL SIGNS**	**Type of intoxication suspicion**	**Observations**
FEB.	**1**	SB	Small, blackened bees with exteriorised proboscis, wings stretched at 90 degrees, high mortality	**Drugs intoxication**	The irrational use of various external antiparasitic substances
APR.	**5**	GR, GR, PH, IF, VS	Depopulation, diarrhea, constipation, bloating, bees with the proboscis pulled out, exteriorised needle	**Toxic food intoxication** (pollen)	Without Nosema (after rain)
MAY	**8**	SB, GR, IF, DB, DB, SB, SM, AG	Blackened bees pulled out at the entrance of the hive, exteriorised needle, blackened head in the cell, uncoordinated movements, paralysis, depopulation, dead bees with crowded legs, wings stretched at 90 degrees, exteriorised proboscis, reduced activity in the hive and outside, paresis, mortality	**Chemical intoxication** (pesticides, mosquito sprays, etc.)	(after rain)
JUL.	**4**	CV, TL, IF. GR	Depopulation, small and blackened bees, abdominal bloating, diarrhea	**Toxic food intoxication** (linden pollen)	Without Nosema (after rain)

The suspicion of intoxication with toxic food (pollen) was identified in the active beekeeping season during harvesting, after rain, and was characterized symptomatically by: bloating, diarrhea, constipation, exteriorisation of the genital apparatus in drones, pollen glued to the bees' feet, their presence on the flying board, paresis, inability to fly (Figure 2, 3, 4, 5, 6, 7).

Fig. 2. Morphoclinical aspects: bloating of the adult bee's abdomen in the pollen intoxication suspicion

Fig. 3. Intoxication with toxic food – pollen, buckwheat honey (stains of diarrhea on the frames from the hive)

Fig. 4. Pollen intoxication in working bees (wet pollen attached to the bees' feet)

Fig. 5. Pollen intoxication in drones (exteriorization of the genital apparatus)

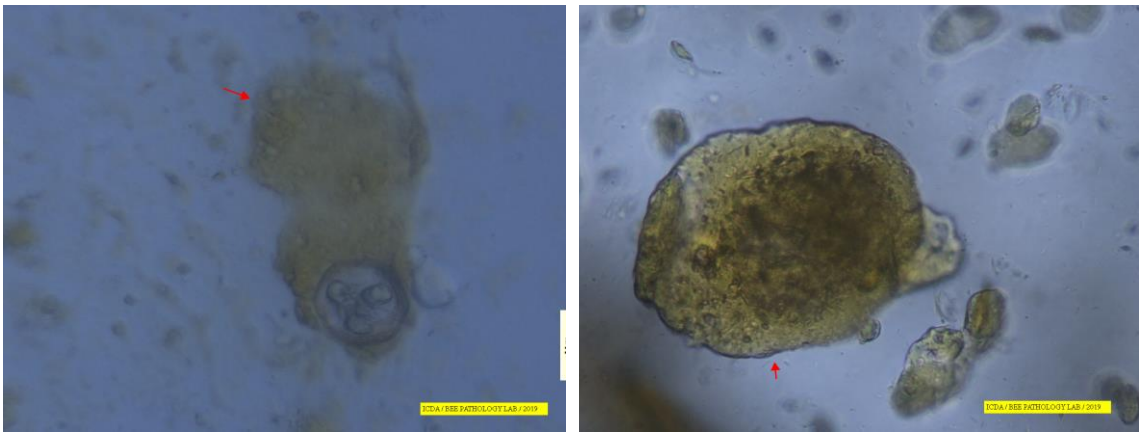


Fig. 6. Diarrheal feces (left) or constipation (right) in the intoxication with toxic food

The suspicion of intoxication with *chemical substances* has been identified in the beekeeping season during harvesting (fruit trees, colza, sunflower, linden), after pesticide and / or mosquito spraying, followed by heavy rains with rain water puddles, but also following the use of some toxic paints for the protection and individualization of the hives, or the beekeepers using artificial combs containing toxic paraffin solvents. (Figures 7, 8)

Fig. 7. Morphoclinical aspects in the intoxication with *chemical substances* (pesticides) after spraying the plant cultures (fruit trees, colza, etc.)

Fig. 8. Morphoclinical aspects in the suspicion of intoxication with chemical substances (blackened bees with the wings stretched at 90 degrees)

The usage of *drugs* without following the indications in the package leaflet and also the use of some medicinal products intended for other animal species without knowing the exact dose of administration in bees, have led to the emergence of brutal drug intoxication cases, shortly after treatments, with a mortality rate of 100% (one apiary in February).

Conclusions

1. Suspicions of intoxication in bees represented 22.57% of the bee families, and mortality rate was 1.88%, accompanied by significant depopulation.

2. The largest share of the suspected intoxications in the active season of 2019 was attributed to the chemical substances intoxication (59.6%), followed by toxic food intoxication (34.07%) and drugs intoxication (6.33%).
3. By educating the beekeepers and following the legislation regarding the prescription of veterinary medicines by specialists, the impact of intoxications on bees can be limited.
4. The intoxication with chemical substances has decreased by applying some administrative and training measures for the beekeepers.
5. Applying some intoxication prevention and *environment protection* measures during the bees' harvesting period represents a good measure to limit the economic losses.

Compliance with ethical standards: The research does not involve human and/or animal experimentation.

Conflict of interest: The authors declare that they have no conflict of interest. We mention that the research conducted has no connection with the activity of official territorial or central laboratories nominated for the monitoring and control of bee diseases.

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The morphological peculiarities of the digestive system in peacock (*Pavo cristatus*)

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Abstract
The peacock's beak has the characteristic aspect as in all granivores, being relatively short, thick at the base and sharpened at the tip, consisting in a curved upper valve which covers the lower valve tip. The ceiling of the oropharyngeal cavity is narrow and covered by two palatine lateral ridges which fuse into a brief median ridge. The palatine fissure appears such as a cleft, aborally placed to the beak's commissure and slightly wider in its posterior half. The tongue is triangular-like and sharpened ended, it follows the shape of the inferior valve but being small in length, it partly fills the floor. The peacock' esophagus is about 25 cm long, the last third of the cervical part represents in fact the crop, globular in shape. Proventriculus appears elongated, fusiform and approximately 3,5-4 cm long. The ventriculus is 6,5 cm long, 5 cm wide and 3-3,2 cm thick. The two ceca are about 20 cm long lying on each side of ileum. The proximal portion is 8 about cm long showing a thicker wall, then follows the tonsillar portion with thinner wall, it appears as a blind sac with a rounded apex. The hepatic lobes are almost equal in size. The left lobe is divided into medial and lateral portion. The pancreas consists of three lobes: ventral, dorsal and splenic.
Keywords*: digestive system, morphology, peacock.*

Introduction

The Indian peacock bears the iridescent blue and green plumage, mostly metallic blue and green, it belongs to *Aves* Class, *Galliformes* Order, *Phasianidae* Family and *Phasianinae* Subfamily.

Peacocks are omnivores, the eat food being mostly represented by plant parts, flower petals, seed heads, insects and other arthropods, sometimes reptiles and amphibians. These birds are not picky and will eat almost anything they can fit in their beak and digest. They usually hunt insects like ants, crickets and termites, millipedes and other arthropods and small mammals (9).

The studies done in this research paper is trying to fill the gaps of information concerning the digestive system in Indian peacock.

Materials and methods

The study was conducted on two corpses, aged 7, male and female, coming from a household, private property, dead after a respiratory disease. By using classical anatomical methods there were noticed the particularities of the segments of their digestive system. Each organ was examined by observing both its dimensions, its topography and eventually any difference regarding other species. For illustration, they were photographed with the Olympus camera, and they were processed in Adobe Photoshop.

Fig. 1 Peacocks in shelter

Fig. 2 Peacock in shelter

Results and discussions

The peacock has the characteristic beak of the granivores, relatively short, thick at the base and sharpened at the tip and consists in a curved upper valve that covers the lower valve tip. The horned sheath (*Ramphotheca*) gets thicker and hardness especially at the tip of the beak. At the base of the upper valve, the pigmented skin has wrinkled appearance and flexible, being known as *ceroma*. On the lateral sides of the upper valve there are the two nostrils which appear as a slot, having triangular aspect (1,2,3).

The lower valve consists of two branches with a sharp peak and being partially covered at the top by the upper valve. The two valves take contact with theirs sharp edges. The oropharyngeal cavity has a narrow ceiling, being covered by two palatine lateral ridges (*Rugae palatine laterales*) which fuse through a brief median ridge, rostral placed (*Rugapalatina mediana*).

Laterally to the ridges, there are found both openings of the maxillary salivary glands (*Ostium gl. maxillaris monostomatica*). Palatine papillae are transversally arranged, into two rows, one row placed on the proximal part of the palatine fissure and the other, halfway through the palatine fissure, more obviously with developed papillae (4,5,6).

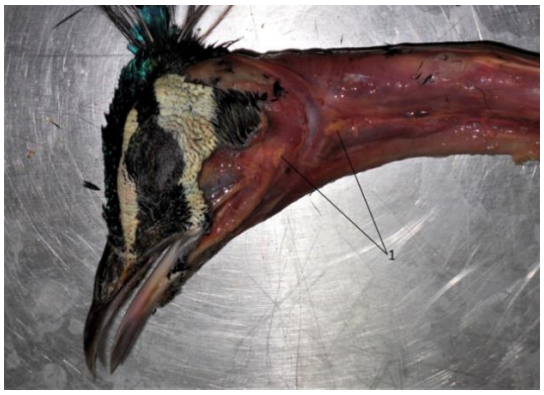

Fig. 3 The aspect of the beak in peacock
1. Gll. mandibulares

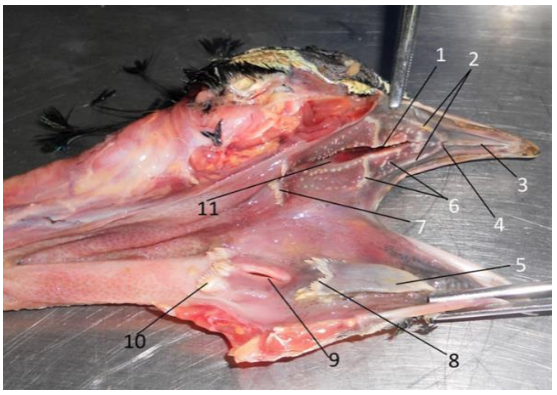

Fig. 4 The aspect of the oropharyngeal cavity in peacock: 1. Choana. 2. Plica palatine laterale. 3. Plica palatina mediana. 4. Ostium glandulae maxillaris. 5. Lingua. 6. Papillae palatinae. 7. Papillae pharingeales. 8. Papillae linguales. 9. Mons laryngealis. 10. Papillae pharingeales. 11. Infundibulum.

The palatine fissure has a cleft appearance, being aborally placed to the beak's commissure, it is wider in its posterior half, accomplishing the communication between the two choana and the oropharyngeal cavity. The two choana are separated from each other by the vomer bone. The lateral edges of the palatine fissure are flanked by a few small conical papillae. It is noticed that the palatine mucosa is rich in small palatine glands which appear as many tubercles that are spread into the mucosa and open through numerous orifices. Behind the infundibular cleft, the oropharyngeal cavity suddenly thickens, the mucosa forms a semilunar crest which resemble a sphincter, being outlined by multiple papillae (*Papillae pharyngeales)* thus delimiting the passage towards the esophagus (2,7,8).

The tongue has a triangle shape, with a sharp apex and it is small in length following the edges of the inferior valve without completely filling the floor. The lingual mucosa is strongly keratinized at the level of tip, whitish in color and hardened when touch it. On the dorsal surface it can be seen the longitudinal median groove, stretching the entire surface. The base of the tongue is marked by a transverse row of large papillae (*Papillae linguales transversales*).

The maxillary salivary glands form two compact flattened masses, laying out on both sides of the median plane, in the rostral portion of the hard palate, the canal of each gland opens through an orifice aborally placed to the beak.

Palatine salivary glands appear such as some tubers spread into the palatine mucosa nearer the edge of the palatine fissure and the sides of the hard palate.

The angular salivary glands are poorly developed, barely noticeable and placed under the zygomatic arches, close to the beak commissures.

The sublingual salivary glands are highly developed in peacock, sacciform in shape, being placed into the intermandibular space, between the cutaneous and the milohyoid muscles. The gland ducts open into the sublingual space (1,2,7,8).

The peacock's esophagus is about 25 cm long. The cervical portion of the esophagus originates dorsally from the larynx through a more dilated funnel-shaped portion. The esophagus runs on the dorsal part of the trachea in its proximal third of the cervical region, then it is placed laterally and on the right side of the trachea, where it makes a convex loop ventrally to the trachea. The last third of the cervical portion of the esophagus represents the crop which is globular in

shape, slightly diverted to the right, cranially to the clavicles placed and takes contact with the pectoral muscle. The thoracic portion of the esophagus is placed dorsally to the trachea and the base of the heart, to the intercostal 3-5 spaces where it opens into the glandular stomach through the cardia. This portion is placed between the both lungs and the cervical and clavicular air sacs (1,7,8).

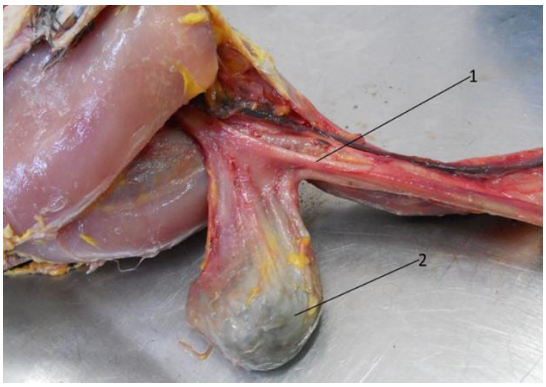

Fig. 5 The appearance of the esophagus and crop in peacock: 1.Esophagus. 2. Ingluvies

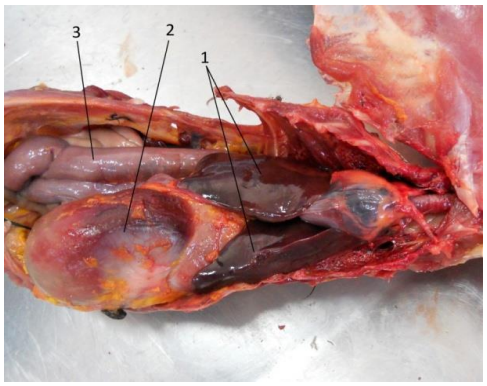

Fig. 6 Theappearance of the thoracoabdominal cavity in peacock: 1. Hepar (lobus hepatis dexter, lobus hepatis sinister). 2. Ventriculus, pars muscularis. 3. Duodenum

The stomach in peacock consists of the two well-defined compartments: the proventriculus and the ventriculus (gizzard). Proventriculus is elongated, fusiform and approximately 3,5-4 cm long. It is placed in the thoracoabdominal cavity, to the left of the median plane, ventrally to the aorta and dorsally to liver. It presents numerous macroscopic elevations (*papillae*) through which pass the collecting ducts from a thick bed of glands, very visible on the internal surface of the wall. In the dorsal area of the proventriculus opens the esophagus through the cardiac opening which is slightly dilated. At this level, the mucosa produces a mucosal fold that marks the cardiac orifice. The transition towards the ventriculus is represented by a narrow tubular space, the isthmus, approximately 0,5 cm long, in fact being the true intermediate portion of the stomach. This portion has no glands and less rigid walls (2,3,5,7,8).

The ventriculus or gizzard is lens-shaped because of his convex surfaces. The cranial blind sac which is dorsally placed, communicates with the gastric isthmus through a wide opening, and the main cavity of the gizzard communicates to the right with the pyloric opening.

The ventriculus has a length of approximately 6,5 cm, 5 cm width and 3-3,2 cm thickness (as a whole). It occupies a large portion on the left side of the thoracoabdominal cavity, slightly exceeding the median plane. On the right side, it takes contact with spleen, duodenum and large intestine. On the left side is in contact with the liver and the wall of the abdominal cavity, where it can be felt through palpation.

The yellow-green, thick, cuticle has plenty of folds.

The lateral, dorsal and ventral muscles are developed, having a dark red color and massive muscle bellies delimiting the lateral edges of the ventriculus. On both sides of the ventricle, the two muscles continue with their strong aponeurosis, with whitish aspect, to form the tendinous center. The intermediate muscles, cranial and caudal, have smaller muscular belly and a lighter reddish color than the others (2,3,7,8).

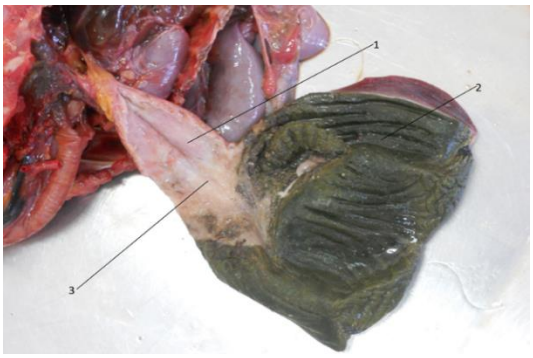

Fig. 7 The inside aspect of the stomach in peacock: 1. Proventriculus, pars glandularis. 2.Cuticula gastris, pars muscularis. 3. Isthmus.

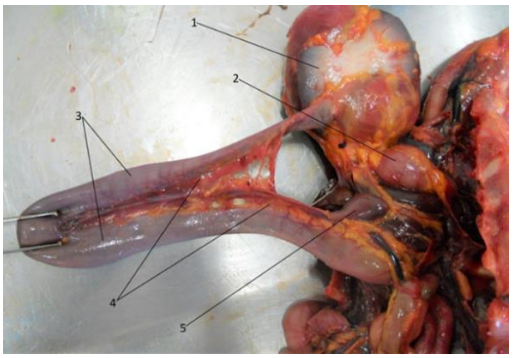

Fig. 8 The appearance of the stomach and duodenum in peacock: 1. Ventriculus, pars muscularis. 2. Proventriculus, pars glandularis. 3. Duodenum. 4. Pancreas. 5. Lien.

The small intestine is made up of three distinct segments, the duodenum, the jejunum and the ileum, and the large intestine consists of two ceca, the colon and the rectum which opened in the cloaca.

The peacock duodenum has the appearance of a U-shaped loop, whose branches are approximately equal lengths of 10 cm which represent its portions, descending and ascending. The descending portion starts from the pyloric opening and sets a caudal orientation. The ascending portion is dorsally placed from the other.

The jejunum is the longest segment of the small intestine and measures approximately 60 cm. It is suspended by the large mesentery and occupies most of the abdominal cavity.

The ileum is a 20 cm long rectilinear segment placed between the two ceca, to which it is bounded by the ileocecal folds. It continues with the colon at the level of the opening between the two ceca.

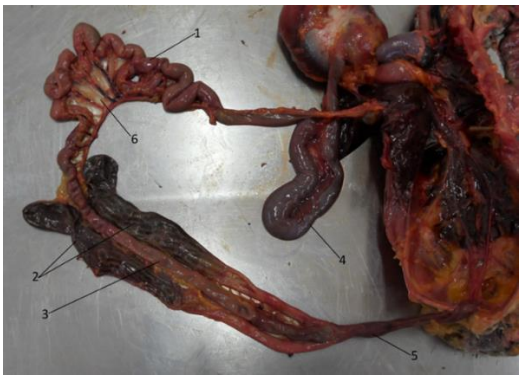

Fig. 9 The appearance of the intestinein peacock: 1. Jejunum. 2. Cecum. 3. Ileum. 4. Duodenum. 5. Rectum. 6. Mesenterium.

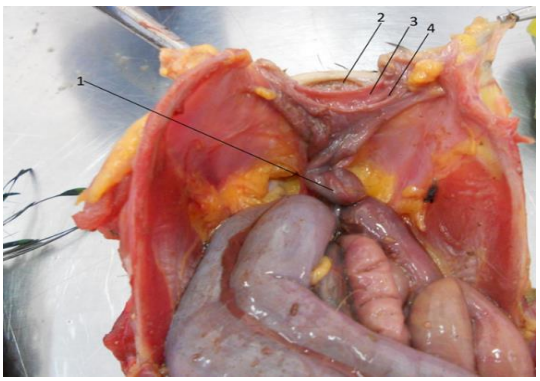

Fig. 10 The inside appearance of the cloaca in peacock: 1. Rectum. 2. Proctodeum. 3. Urodeum. 4. Coprodeum.

The two ceca measure about 20 cm long, each of them are sitting on both sides of the ileum. The proximal portion, the base (*Basis ceci*), has thicker wall and it is 8 cm long. The body

(*Corpus ceci*), that follows the base, has a thinner wall, wider lumen and ends with a rounded apex (*Apex ceci*).

The colon is rectilinear and extremely short, having no demarcation with the next segment.

The rectum, measuring 5 cm, continues caudally the colon in the median plane near the abdominal-pelvic cavity ceiling and opens in the first portion of the cloaca.

Cloaca has its three compartments, coprodeum, urodeum and proctodeum well delimited by the copro-urodeal and urodeo-proctodeal folds. The circular musculature of the cloaca opening, known as *vent*, is strong, structuring the anal sphincter which appears as a sleeve around the orifice.

Inside the abdominal cavity, the liver in peacock is placed almost horizontally, occupying a large portion of the floor. It consists of the right and the left main lobes being separated by two interlobular cranial and caudal notches. The hilus appears as a narrow depression placed on the visceral surface of the liver; it extends over the entire interlobular portion and partly on the liver's lobes. The hepatic lobes are almost equal. The left lobe is divided into a medial and lateral portion. The left middle lobe is separated from the gallbladder by a wide and deep notch, up to the hepatic hilus. On the visceral face there are secondary notches which divide the hepatic parenchyma into the reduced papillary lobes, near the liver hilus placed.

The pancreas is pink in color, being located inside the duodenal loop, between the duodenal-pancreatic ligament layers. It consists of three lobes: ventral, dorsal and splenic. The ventral and dorsal lobes are approximately equal having the same length. The splenic lobe is located near the spleen and is poorly developed, being embedded in a fat mass (2,3,7,8).

Conclusions

The peacock has the beak adapted to the omnivorous diet, relatively short, thick at the base and sharpened at the tip and consists in a curved upper valve that covers the lower valve tip.

The peacock esophagus is about 25 cm long with the last third of the cervical portion representing the crop, globular in shape, spacious and large for temporary storage of food, characteristic for the omnivorous diet, slightly diverted to the right, anterior to the clavicles and in contact with the pectoral muscle.

Proventriculus has an elongated aspect, fusiform and a length of approximately 3,5-4 cm.

The ventriculus has a length of approximately 6,5 cm, 5 cm width and 3-3,2 cm thickness (as a whole). It occupies a large portion on the left side of the thoracoabdominal cavity, slightly exceeding to the right, the median plane of the cavity.

The peacock duodenum has the appearance of a U-shaped loop, whose branches are approximately equal lengths of 10 cm and represent its portions, descending and ascending.

The two ceca measure about 20 cm long, each of them are sitting on both sides of the ileum. The base has thicker wall, stretching 8 cm and the body has a thinner wall, wider lumen and ends with a rounded apex.

The hepatic lobes are almost equal size, with the left lobe divided into a medial and one lateral portion.

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Estimation of laparotomic incision lengh in corelation with uterine size

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Abstract
The objective of this article is to find a way to estimate the lengh of the laparotomic incision after the size of the organ that needs to be removed was measured using an ultrasound machine. Incision size is a very important aspect in post-surgery recovery. A small incision leads to a faster recovery, reduces the risc of post-surgery infections, it leads to less pain for the animal and a lesser inflamatory response. An incision made at first too small needs to be lengthened until it gets to the proppers size and that means extending the time of surgery, producing unnecesary trauma by makeing multiple cuts and increasing the risk of error. We used a total of 25 queens and 18 bitches for this study. We performed an ultrasound exam on each animal and determened the size of the uterine horns and ovaries. Than we used the area of the largest section to estimate the lengh of the incision.
Keywords*: laparotomy, ovariohiyterectomy, ultrasound, incision size*

Introduction

The use of ultrasound exam is more often than ever found in private practices for diagnosing various abdominal organs abnormalities. Ventral median celiotomy and ovariohisterectomy was compared to laparoscopic ovariohisterectomy and laparoscopic ovariohisterectomy showed a faster recovery (Culp WT,2009). On the other hand, the lack of minimal invasive surgery set-ups makes the laparotomy the go to method for most of the surgeons. Laparotomy being the prefered method it is important to know at first how long the incision should be.

Reviewing the literature we concluded that the cases where laparoscopy was used for ovariohisterectomy when the uterus was enlarged were seldon. For this reason linea alba laparotomy is the best way to remove an enlarged uterus or ovaries. Laparoscopic asisted ovariohisterectomy can pe used in cases of pyometra but require a cerfull selection of the cases and gentle handling in order to avoid complications la uterine rupture(Adamovich-Rippe KN 2013).

A smaller incision has more benefits than a larger one, we considered that it is important to estimate the optimal size of the incision. The most important benefits are lower incidence of infections, reduced pain and faster recovery(Brenda Austin,2003)

Materials and methods

A total of 25 queens and 18 intact female dogs were selected for this study. All animals were selected after we found an enlarged uterus because of gestation or because of inflamatory disease. The queens were of age 7 months to 10 years and the intact female dogs were 2 years to 6 years old.. All animals were brought in by their owners either for an exam or for spaying.

At first all animals were clinically examined by using inspection, palpation and thermometry. We than proceded to do an ultrasound exam. During the ultrasound exam we identified the uterine horns enlarged due to pregnancy or liquid acumulation inside the uterine horns. During the ultrasound scanning we measured the diameter of the uterine horns and the area in their wides point. Then we divided the diameter uf the uterus by 2 and multiplied the result by 3.14(π), thus obtaining the lengh of the incision.

To reach this conclusion we considered the fact that in section, the uterine horns form a circle. The circle circumference being 2 times π times radius, we considered that the lenght of the incision is the circle circumferince divided by 2, thus the lengh of the incision beeing π times radius.
Circle circumferince=2·π·radius
Lengh of incision=circle circumferince / 2
Lengh of incision= π·radius

Another way to determine the lengh of the incision for cases were the utherine body was not perfectly circular was to determine the area, extrapolate and do the calculations as it would be a perfect circle.

In cases were the area of desired formation was irregular, we used the ultrasound machine to determine it's area and the sqare root of lengh of the incision is π times area. This formula was obtained by considering the formula above square root raised at the power of square.
$(\text{Lengh of incision})^2=\pi \cdot A \Rightarrow \text{Lengh of incision}=\sqrt{\pi \cdot A}$

All animals were placed in dorsal recumbercy and imobilised after they were anesthetized with xylazin and ketamine. The ventral abdomen was shaved before we performed the ultrasound exam. We desinfected the abdomen and than we placed a sterile field. We calculated the lengh of the incision based on formula and proceded to surgery. In two cases because the uterus could not be folded to be extracted we had to lenghted the incision. In this two female dogs the thickest part of the uterus showed first in the abdominal acces and we could not identify a thinner section of the uterus or the ovaries in order to not have to fold the uterine horns.

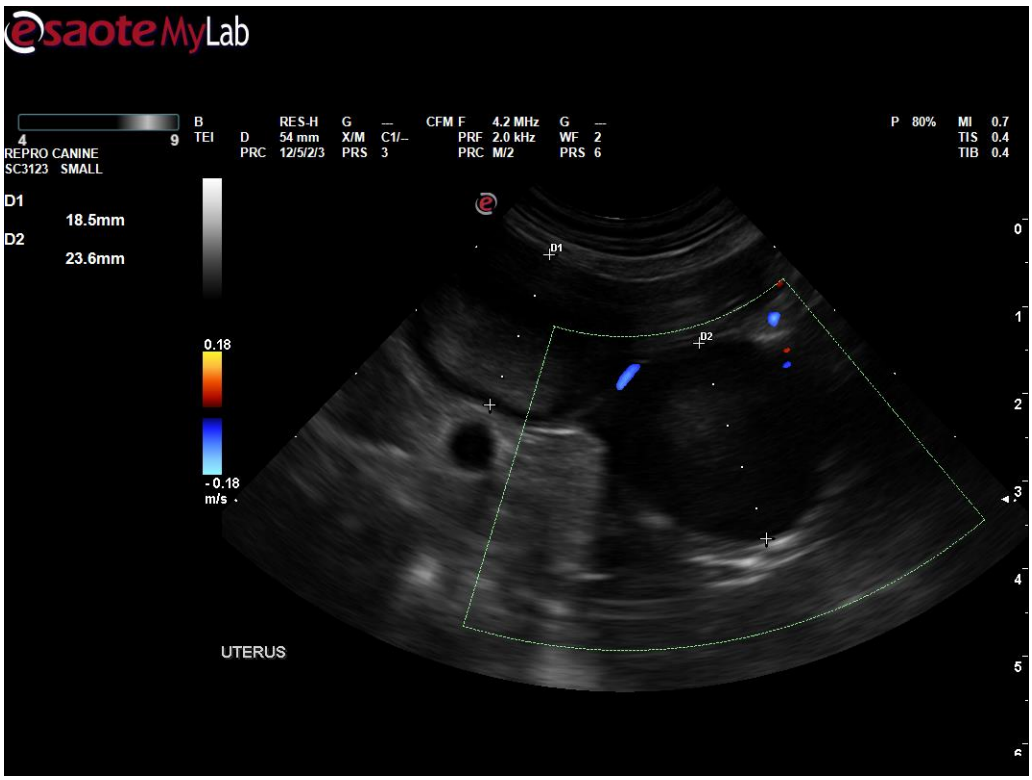

Fig. 1. Female dog, 4 years old. Uterine horns are dilated and contain both hypoecogenic and hyperecogenic areas. The wides area is 23.6 mm in diameter.

In figure 1 we examined the uterine horns using the ultrasound machine. We measured the diameter of one uterine horn in it's wides area and it measured 23.6 mm. The uterine horn beeing of approximate circular form we used the formula discussed before and divided 23.6 mm by 2 and obtained the radius of the circle that was 11.8 mm. We than multiplied this number with π and obtained 37.05 mm.

When we performed surgery the lengh of the incision was 37 mm. We managed to safely remove the uterus. The lengh of the incision sutured is shown in figure 2.

We proceded the same for all the animals included in this study and in almost all situations.

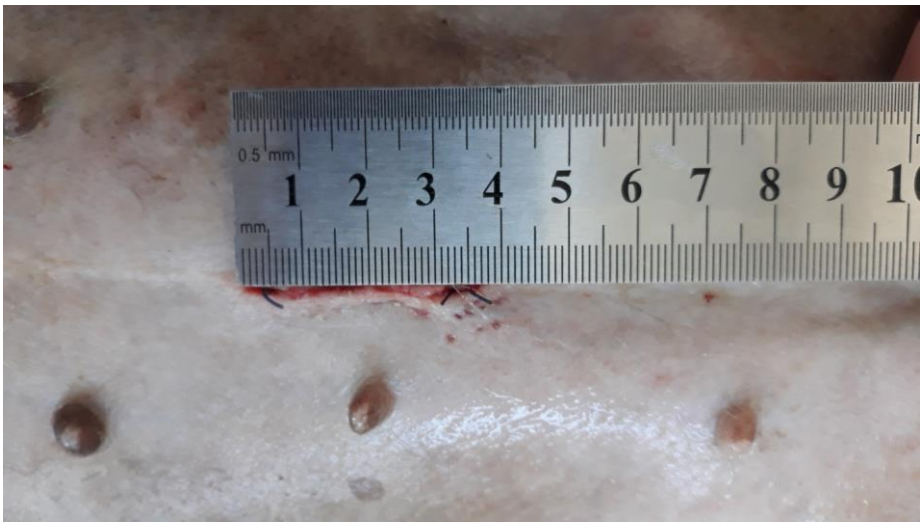

Fig. 2. Female dog, 4 years old. Lengh of incision is 37 mm.

Discussion

Laparotomy is a procedure that is regularly used in animal practice. It is the most used method for spaying bitches and queens and also for solving distocies, pyometra and aother uterine patologies.

Considering this, it is advised that the incision to be made as small as posible in order to ensure a faster recovery. Other benefits of small incisions are reduced pain, smaller chances of infections and other post-surgical complications.

On the other hand an incision too small increases the risk of accidents or makes the procedure impossible. The solution to this problem is extending the incision line but this means wasted time and materials.

We concluded that we needed a way of determening the lengh of the incision before we started surgery. After reaching the formula lengh of incision is πtimes radius of the circle that is described by the uterine horn we aplied it in practice.

The estimated lengh was correct. Considering that all anatomical structures involved have a certain degree of elasticity, this works further more into our advantage. Linea alba is formed of connective tissue and permits a small degree of elasticity. On the other hand, the uterus is elastic and allows to be folded or compressed in order to fit through the incision. This is harder to achive with pregnant uteruses because fetuses tent to stay in place inside a uterus. In pregnant animals we identified the ovaries first and starting from the ovary we pulled the uterine horns and body out in order to ligaturate it.

Concusions

A basic rule in surgery is that incision should be long enough in order to provide propper acces to the area of pathology and to be confortable for the surgeon.

Incision should provide propper acces to the area of interest. Considering that a smaller incision behaves better than a long one, estimating it's lengh before surgery commences it's a factor that betters the odds of a good recovery.

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Inguinal herniated pregnant uterus in a 3years old common breed bitch. A case study

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Abstract
A 3 years old bitch was admitted with an inguinal mass. The ultrasound exam determined that the mass contained one live fetus and another 2 hyperechoic areas that surrounded one hypoechoic area each. We made an incision on the mass and found both uterine horns herniated through the left inguinal ring. We decided to perform ovariohysterectomy through the inguinal ring and after we did herniorrhaphy. The bitch recovered in a few days.
Keywords*: ovariohysterectomy, herniated uterus, gestation*

Introduction

Inguinal hernia in female dogs is a seldom patology. Usually in ocurs more often in female dogs than male dogs and more often in middle aged dogs. In female dogs the inguinal canal is both shorter and wider in diameter than in male dogs. Also, sex hormones, especially estrogen, might be involved in pathogenesis of inguinal hernia because most cases were reported in female dogs during estrus or pregnancy and have not been reported in neutered females(Smeak DD, 1993).

The size of a hernia is usually assessed visually and by palpation. The content of the hernial sac can be determined by papation, radiography and more precise by an ultrasound exam.

Materials and method

A 3 years common breed female dog was brought into our clinic because it had an inguinal mass on the left side. The owners said that about 6 months ago the dog had a mass on the same side and it grew for a while, but before they got the chance to visit a veterinarian the mass went away. The owners could not give us any information about her previous cyclic phase. They also said that the dog was not neutered.

During the clinical exam the dog acted normal and no abnormalities were identified. The owners said the female dog is eating, it's feces are normal, it has no urinary disturbance.
We decided to do an ultrasound exam. During the ultrasound exam we identified a live fetus and another 2 hypoechoic areas surrounded by a thick hyperechoic area. The fetus was older than 30 days. In Fig 1 there is the live fetus. We used Doppler ultrasound to see if it was alive. On the ultrasound we saw that the heart was beating and we also managed to identify the aortic artery and vena cava of the fetus. In Fig 2 we showed the intensity of the vascularization of one of the masses. The ultrasound exam suggested there are inflammatory processes present.

We decided to perform ovariohysterectomy to remove the herniated organ. The bitch was anesthetized using Xylazine and Ketamine. It was placed in a dorsal recumbency and prepared for surgery. We made a first incision on the skin in the left inguinal area. Because the herniated uterus could not be placed back into the abdominal cavity, we decided to perform ovariohysterectomy through the inguinal ring. In Fig 3 there is the uterus that could not be placed back into the abdominal cavity. The broad ligament was dilacerated, we identified the left ovary, we clamped the ovarian pedicle and then we made a vascular suture on the pedicle. We dilacerated the other broad ligament until we found the other ovary, we put a clamp on the right ovarian pedicle, we then sutured the pedicle and then cut the pedicle. In the end we sutured the uterine body following a textbook technique.

Post-surgery we administered antibiotics for 5 days and the stiches were removed 10 days post-surgery.

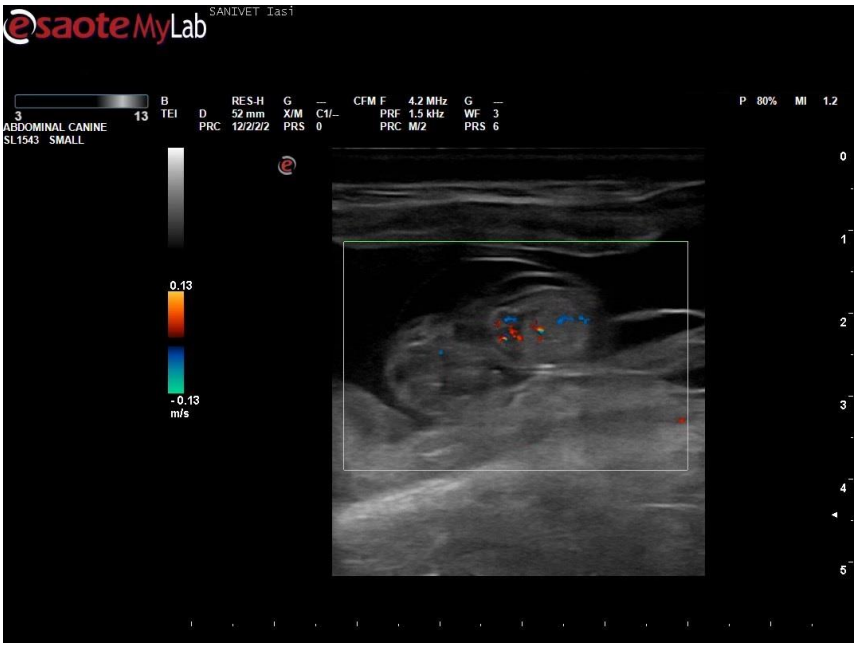

Fig. 1. Female dog, 3 years old. Pregnant herniated uterus. Doppler used to show the heart beating

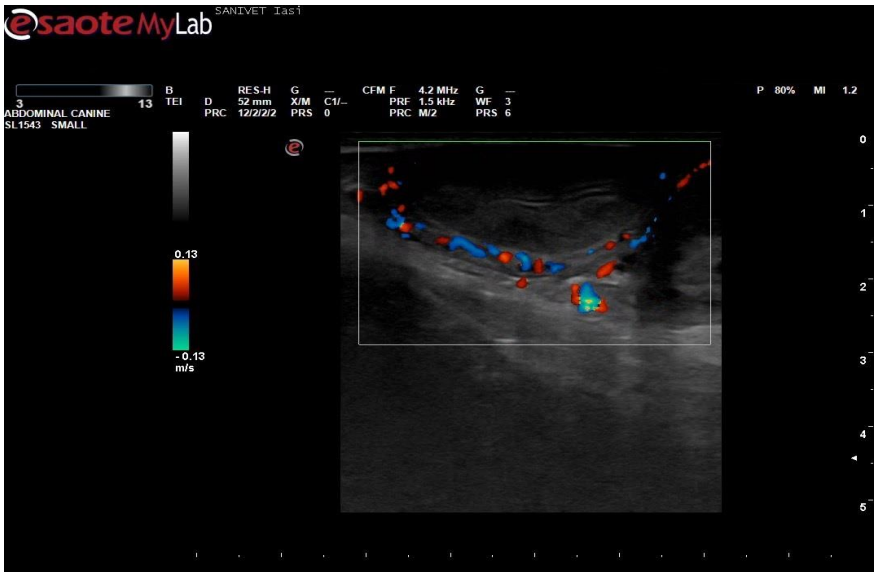

Fig. 2. Female dog, 3 years old. Pregnant utherine masses. Doppler used to show the vascularization of the other 2 uterine masses

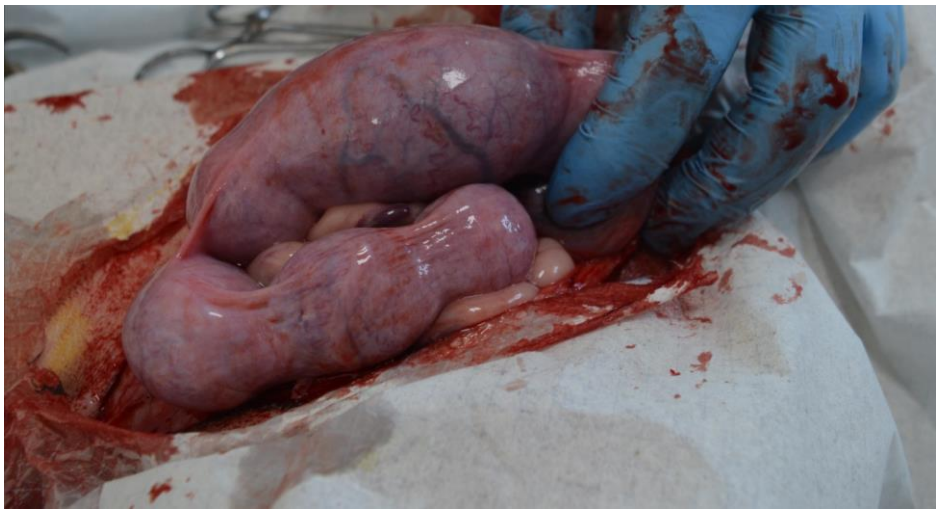

Fig. 3. Female dog, 3 years old. Herniated pregnant utherine horns.

Discussion

Inguinal masses may be of different origins: inguinal hernia, inguinal lymph node hypertrophy, mammary gland tumor, abscesses, hematoma or lipoma. By palpating the mass we were able to distinguish tubular shaped formations that were distended in some areas. The ultrasound exam helped us to determine that organ herniated was a pregnant uterus.

We consider that the main cause for the herniated uterus is the anatomical one because the inguinal ring was loose. There was also a herniated ovary. Another argument for the inguinal ring being too loose is that we managed to perform ovariohysterectomy by pulling the ovaries through the inguinal ring.

This is a novelty because we did not find in literature another case whereovariohysterectomy was performed in a pregnant bitch through the inguinal ring. In literature we identified similar cases especially were the pathology was pyometra with the uterus herniated. In this case the uterus was placed back into the abdominal cavity and ovariohysterectomy was performed as usual through the linea alba or the herniated uterine horn was sutured, then the herniated part was removed, the remainings were placed back into the abdominal cavity and ovariohysterectomy was than performed through the linea alba.

During the ultrasound exam beside the fetus we identified another 2 intrauterine formations that were hyperechogenic and had a hypoechogenic center. We assumed that there were 2 embryos that had died and now they were reabsorbed. We also assumed that the cause of death for the embryos was the lack of proper blood perfusion caused by compression on blood vessels when the uterus increased in size. Uterine ischemia effects were studied in rats where it showed growth retardation(Thaete and Neerhof 2006). This phenomenon takes place because during alternation periods of ischemia and proper perfusion leukocytes accumulate into uterus and they amplify the pathologic processes that took place due to ischemia (Miyakoshi K, 2001).

Improper blood perfusion slows down the reabsorption processes and reduces uterine motility. This means that parturition would have been dystocic, being impossible for the fetus to fit back through the inguinal ring into the abdominal cavity and had to be expelled.

The histopathological examination of figure 4 confirmed the blood stasis in the myometer. In this picture you can see destined vessels loaded with asphyxic blood.

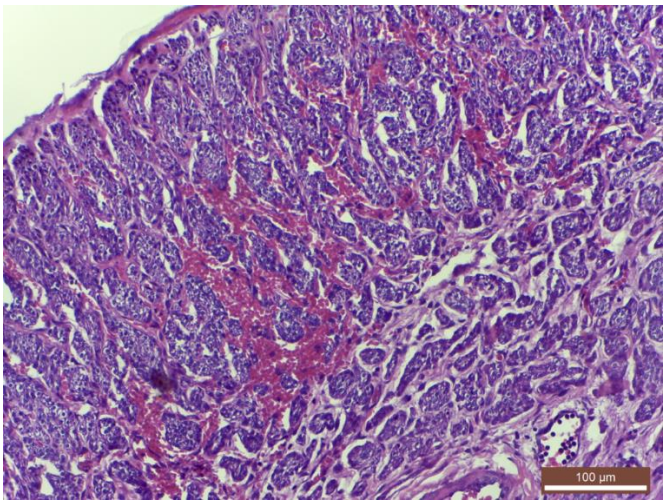

Fig. 4 Female dog 3 years old. Myometer with asphyxic blood stasis in destined vessels.Col. trichrome Masson

We did not consider medical treatment because the only way to repair inguinal hernia is surgery. We also took into account the fact that the size of the uterine horns was too great to fit back through the inguinal ring thus placing back the uterus was impossible. Widening the inguinal ring was possible but it presented great risks because during parturition it was possible for the suture to break loose.

The histopathological exam showed that ovariohysterectomy was the proper way to manage this case. Figure 5 shows hemorrhage, blood stasis and hemosiderophages which suggest chronic blood flow perturbance. Figure 6 show necrosis and puss due to cellular death. Severe endometrial degeneration can be explained by alternation between ischemia and blood perfusion periods. This phenomenon amplifies cellular death due to cytotoxic substances and due to interaction between neutrophils and endothelial cells(Cerqueira NF,2005).

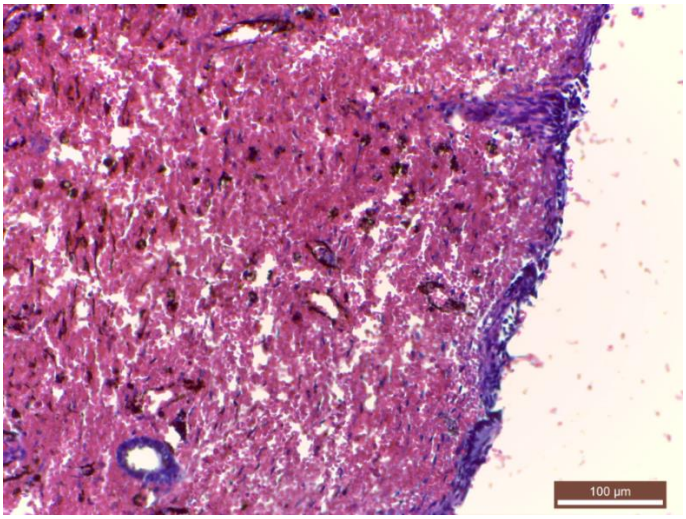

Fig 5. Female dog 3 years old. Myometer. Blood stasis, hemorrhage and hemosiderophages Col. trichrome Masson.

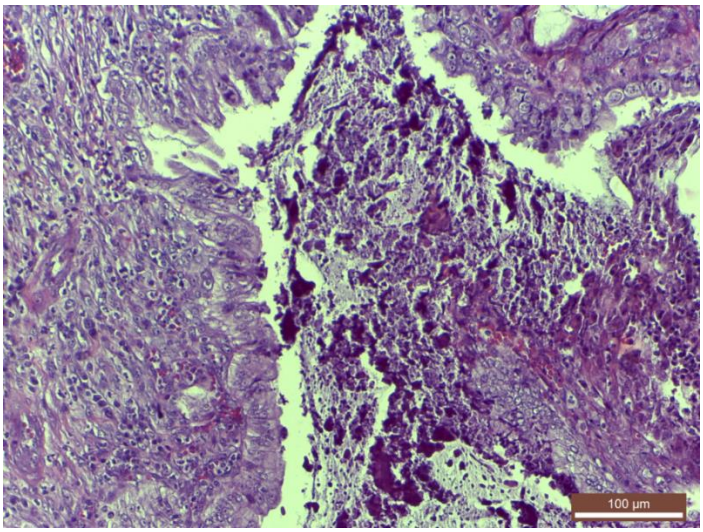

Fig. 6 Female dog 3 years old. Endometrial degeneration.
Necrosis and puss inside uterine lumen. Col. tricrom Masson.

The inguinal hernia was fixed by suturing the margins of the inguinal ring together.

Conclusions

In some entire female dogs—due to a large inguinal ring and favorable hormonal factors, when the uterus increases in size(gestation, liquid accumulation) it could herniate. Moreover, the increase in intraabdominal pressure can also lead to herniation. This case is outstanding because the patienthistory suggests that its last gestation also presented with a herniated uterus and a possible spontaneous abortion since the owner noticed the herniated uterus and one day it just went away. It is also remarkable that the fetus is atleast 30 days old.

Due to the late gestational age it was impossible for the uterus to be placed back inside the abdominal cavity. Considering the ultrasound exam and the histopathological exam, most likely this gestation could not have been carried to full term. Also, considering that it was not a purebred dog we determined that ovariohysterectomy was the right choice in order to prevent further complications.

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Microscopical study regarding the arrangement of tongue muscle in Chinchillas (*Chinchilla lanigera*)

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Abstract

The tongue in mammals is essential for food consumption, oral transport, swallowing, emesis, respiration, coughing. Depending on species, the morphological features of the tongue vary according to the lifestyle and type of diet. The aim of the study was to present the main microscopical features of the tongue in Chinchilla. The biological material was represented by 5 Chinchillas. Longitudinal fragments were collected from the tongue, fixed in the Stieve solution, histologically processed by paraffin technique and stained later by Goldner's trichrome method. In Chinchilla's tongue, the myofibers are oriented longitudinally, transversally, vertically and occasionally in an oblique way. The myofiber bundles are arranged in a superficial dorsal layer, a ventral layer, while the central axis of the organ occupies the space between the two superficial layers. The superficial dorsal layer extends from the tip of the tongue to the intermolecular protuberance. It is thin and plexiform on most of the free portion of the organ but gradually thickens towards the body of the tongue where the cells are predominantly longitudinally and partially oblique. The ventral superficial layer is relatively thin in the free portion of the tongue, consisting mainly longitudinal and scattered vertical or horizontal cells. In the body region of the tongue, the ventral superficial layer is thicker and arranged in two oblique layers (i.e., ventral and dorsal). The central axis of the tongue includes myofibers with a vertical and horizontal orientation. The specific layout of myofiber bundles in Chinchilla's tongue could be related to the specific way of food prehension, mastication and oral transport of feed in this species.

Keywords: *chinchillas, musculature, tongue*

Introduction

Chinchilla, a small-sized rodent, is taxonomically placed in the Mammalia class, Theria subclass, Eutheria clade, Rodentia order, Hystricomorpha suborder, Chinchilidae family (Martonos et al. 2015). The tongue in mammalian is very important in the food consumption, oral transport, swallowing, emesis, respiration, coughing and in humans in speech production (Iwasaki, 2002; Sokoloff and Burkholder, 2012).

The tongue has a muscular - conjunctive axis covered by tongue mucosa. The tongue muscles are formed by striated skeletal cell muscle whit a three-dimensional disposition (Abayomi et al., 2009; Miclăuș et al., 2017). The tongue musculature is divided in 2 main groups: intrinsic muscles and extrinsic muscles (Mireșan, 2009). The extrinsic muscles of tongue are *stiloglosus*, *hyoglossus* and *genioglossus*. The intrinsic musculature of the tongue is represented by the muscle *lingualis proprius* divided in 4 groups: *longitudinales superficiales*, *longitudinales profindi*, *transversi linguae*, and *verticales linguae*. The *stiloglosus* retracts tongue backward and upward, the *hyoglosus* make dorsal surface convex and depresses sides of tongue and the *genioglossus* makes dorsal surface concave and protrude tip of the tongue. By the contractions of the intrinsic musculature is responsible for printing the various forms of the language (Stan and Martonos, 2016). It has also been reported that each muscle indicated a general pattern of muscular activity during swallowing, and each individual had his own swallowing pattern (Yoshioka et al., 1979).

Depending on the species, the morphological characteristics of the tongue vary according to the lifestyle and type of diet (Yanping et al., 2016). A number of studies assessed the morphological characteristics of the mammalian tongue (Kobayashi et al., 2004; Jackowiak, 2006) and the microstructure of the tongue in rodents (Liu and Lee, 1982; Iwasaki and Kobayashi, 1987; Sato et al., 1988; Kobayashi, 1990; Iwasaki et al., 1996; Iwasaki, 2002; Park and Lee, 2009). The rodents are by far the largest order of mammals with over 2000 living species in about 30 families (Abayomi et al., 2009).

In the scientific literature investigated by us, we did not find any detailed information about the disposition and proportion of muscle cell fascicles in the tongue Accordingly, a study of the three-dimensional arrangement of muscles in the Chinchilla's tongue was done.

Materials and methods

The biological material was represented by 5 Chinchilla males aged 1 year and 6 months, clinically healthy. The animals originated from a farm located in Salaj County and were sacrificed in a slaughterhouse for their fur. Immediately after stripping the skin, 4 mm thick longitudinal fragments were taken from the tip to the base of the tongue. The harvested fragments were fixed in the Stieve mix and histologically processed by inclusion in paraffin. Sections with a thickness of 5 μm were performed and colored by Goldner's trichrome method. The preparations were examined under the optical microscope (Olympus BX41) and photographs were taken (Olympus E330) and digitally processed (Adobe Photoshop CS2).

Results

In the free portion of the tongue, the arrangement and proportion of the muscle cells differ from one region to another. Thus, at the tip of the tongue, muscle cells have a plexiform layout and low density. In relation to the connective tissue, they occupy more than half of the section area. In the anterior third zone of the free portion of the organ, the muscle cells are disposed in a superficial dorsal layer and ventral one. The superficial muscular layer is formed by small plexiform fascicles. In the ventral superficial layer, the fascicles are predominantly longitudinal. The central area is occupied by bundles with a vertical and horizontal layout. Vertically oriented fascicles represent the majority (Fig. 1).

In the middle third of the free portion of the tongue, in the superficial dorsal layer, the plexiform layout gradually diminishes and the longitudinally oriented fibers become better represented. In the ventral superficial layer, the arrangement is similar to the one found in the first third but becomes slightly thicker. In the center of the middle third of the free part of the tongue, the ratio is 1:1 and towards the boundary between the middle third and the last third of the free portion, the fibers with the transverse orientation are dominant (Fig. 2, 3).

In the posterior third portion of the free part of the tongue, the dorsal superficial layer is almost 3 times thicker than the middle third. As for the ventral superficial layer, it becomes thicker. It is almost 3 times thicker than the previous third of the free portion. In this layer, the longitudinally oriented fibers prevail. In this area, in the connective tissue between the muscle bundles, the adipocytes are relatively well represented. Most adipocytes are grouped as small adipose lobules (around 20 adipocytes/group; Fig. 4, 5).

At the level of the body of the tongue, the two superficial (dorsal and ventral) parts of muscle cells can be identified. From the level of intermolar (lingual) protrusion, the superficial dorsal layer becomes step by step thinner until it disappears. In the superficial dorsal layer, from the boundary between the free portion and the body of the tongue towards the intermolecular protuberance, the number of cells with longitudinal orientation decreases gradually. As the

longitudinally arranged fibers diminish, the fibers with vertical and transverse orientation become better represented. The ventral superficial layer appears mainly from longitudinally arranged fibers. From place to place there are small and rare muscle bundles with vertical orientation.

The central area between the two superficial surfaces is occupied by fibers with vertical and transverse layout. In the dorsal half of this layer, muscle tissue is a major one. At this point, the ratio of the oriented and vertical arranged fibers is approximately 1:1. In the ventral half, the ratio of muscle as compared to connective tissue is approximately 1:1. In this area, the fibers with a vertical orientation are dominant, while the transverse fibers are few. Connective tissue shows numerous adipose lobules (Fig. 6, 7).

At the level of the intermolare protrusion, the dorsal superficial layer is no longer present so that the fibers present in the central area extend to contact with the lamina propria. In the previous half of the intermolecular protuberance, the fibers with a vertical orientation are more numerous than those oriented transversally.

In the posterior half of the intermolecular protuberance, in the dorsal part, the fibers are predominantly vertical and come in contact with the lamina propria. The muscle tissue does not occupy the majority of the section, because there are many fat lobules among muscle bundles. In the ventral half of the posterior portion of the intermolare protuberance, the muscle tissue is dominant. The ratio of the vertical and cross-layered fibers is approximately 2:1. In the ventral superficial layer, the fibers in the deep plane are slightly oblique to the dorso-cranial / ventro-caudal orientation, while the ones disposed superficially in this layer have opposite layout, with a ventro-cranial / dorso-caudal orientation (Fig. 8, 9). In the dorsal half of the root of the tongue there are numerous glandular acini, which form separate groups of vertically, obliquely or longitudinally oriented muscle cell bundles. In some areas, the acini are so numerous that they occupy more than half of the section area. In the cranial portion of the lingual root, the acini are exclusively serous and are grouped around the circumvallate papillae. From the central part of the root, the muscles bundle go up to the base of the circumvallate papillae. They are slightly obliquely oriented dorso-cranial / ventro-caudal. After the area occupied by serous acini, on the basis of the root of the tongue there is another area occupied exclusively by mucous acini in a very large number.

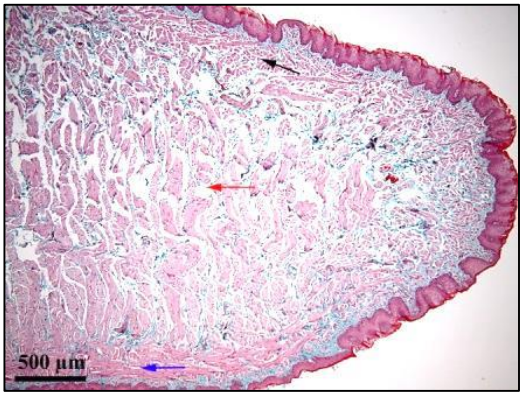

Fig. 1 Anterior third of the free portion; black arrow - dorsal superficial layer; blue arrow - superficial ventral layer; red arrow - central layer (Goldner's trichrome stain).

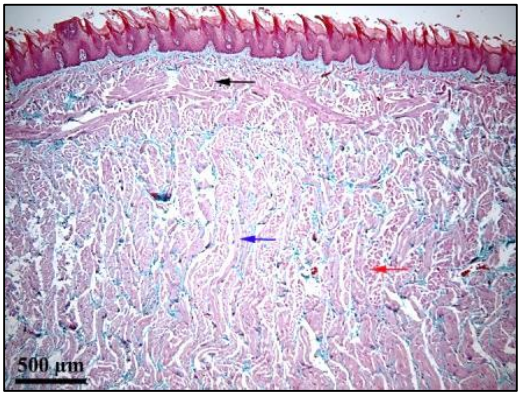

Fig. 2 The middle third of the free portion - the dorsal half; black arrow - dorsal superficial layer; blue arrow - vertical fibers - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

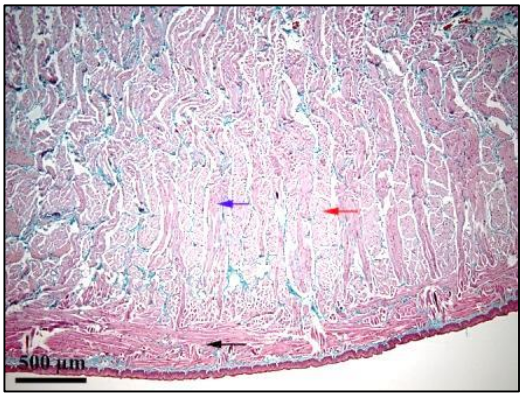

Fig. 3 The middle third of the free portion - the ventral half; black arrow - superficial ventral layer; blue arrow - vertical fibers - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

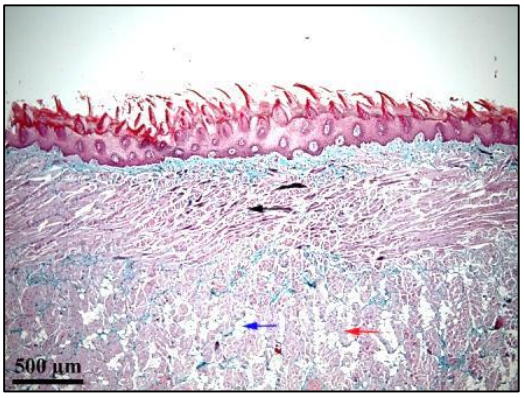

Fig. 4 The posterior third of the free portion - the dorsal half; black arrow - dorsal superficial layer; blue arrow - adipose lobes - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

In the ventral half part of the root, the vertically oriented muscle fibers have a trace approximately perpendicular to surface of the tongue, and extends to the fibers of the ventral superficial layer of the intermolare protuberance area. Among these, there are transversal fasciculi and the ratio of which is about 1: 1 (Fig. 10).

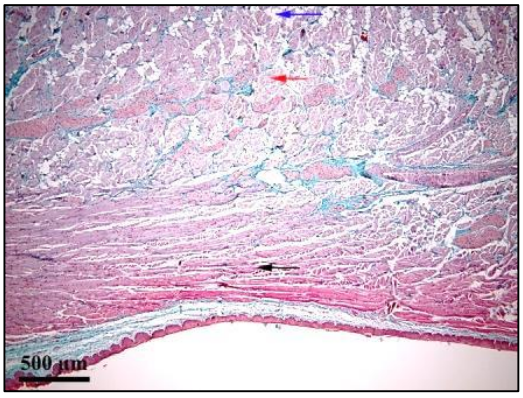

Fig. 5 The posterior third of the free portion - ventral half; black arrow - superficial ventral layer; blue arrow - adipose lobes - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

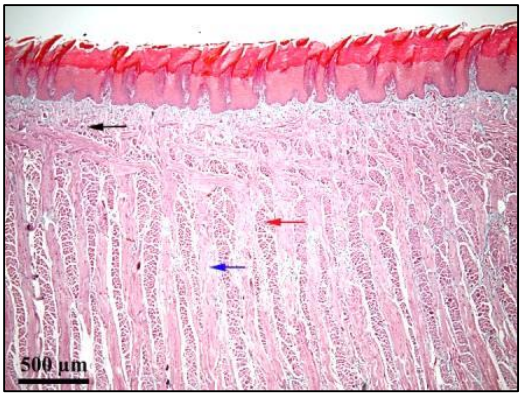

Fig. 6 Anterior half of body of tongue - dorsal half; black arrow - dorsal superficial layer; blue arrow - vertical fibers - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

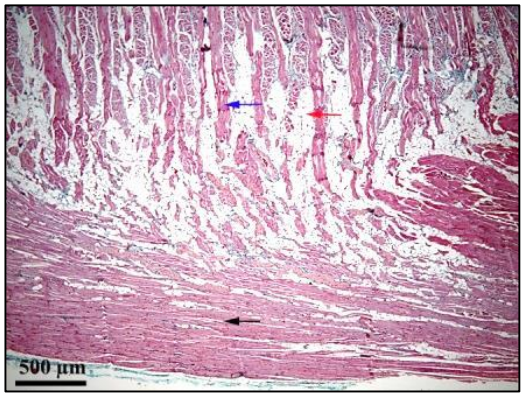

Fig. 7 Anterior half of body of tongue - ventral half; black arrow - superficial ventral layer; blue arrow - vertical fibers - central layer; red arrow - adipose lobes - central layer (Goldner's trichrome stain)

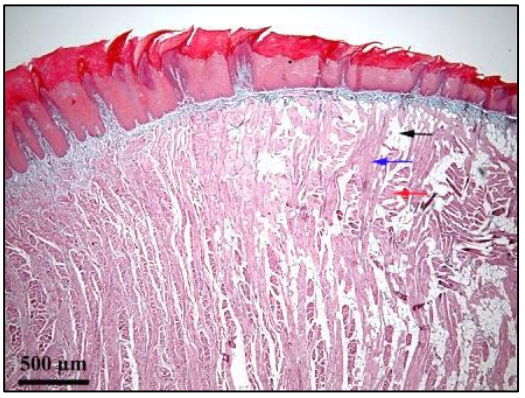

Fig. 8 Intermolar protuberance - dorsal half; black arrow - adipose lobes; blue arrow - vertical fibers - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

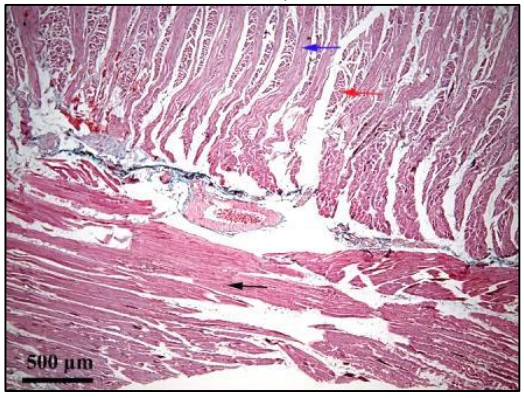

Fig. 9 Intermolar protuberance - ventral half; black arrow - superficial ventral layer; blue arrow - vertical fibers - central layer; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

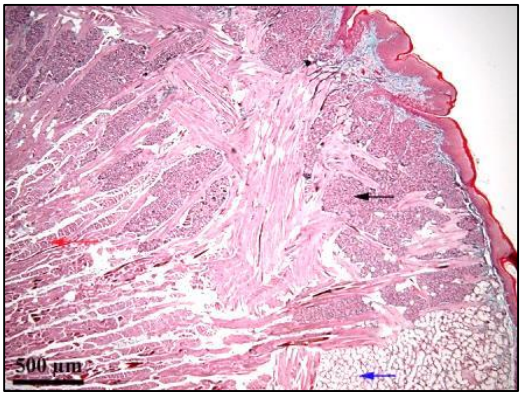

Fig. 10 Root of the tongue; black arrow - serous acini; blue arrow - mucous acini; red arrow - transverse fibers - central layer (Goldner's trichrome stain)

Discussions

The muscular component of the Chinchilla's tongue is represented by longitudinally, transversally, vertically and sometimes obliquely striated myofibers. The arrangement of myofibers differs from one area to another. In the superficial area of both dorsal and ventral sides of the tongue, the myofibers are predominantly longitudinally oriented. The area of the two superficial planes is occupied by predominantly vertical and horizontal cells along with a small number of cells with a different orientation.

At the tip of the tongue, the muscle cells form generally plexiform bundles. Also, the density of myofibers is smaller than in the following portions, occupying more than half of the sectional area. The plexiform layout provides mobility in all directions but at the expense of the contraction force, the amplified aspect and the lower density of the muscle cells as comparing to the next portions. These aspects suggest that the tip of the Chinchilla tongue presents complex mobility but limited force.

The two superficial layers are not identical in terms of thickness and orientation of the muscle cells. Thus, in the first portion of the dorsal superficial layer, the cells lay in a similar fashion to those at the tip of the tongue, but the cell density is somewhat higher. This plexiform layout is maintained up to the posterior third of the free portion of the tongue, from where this layer becomes much thicker. In the first zone of the posterior third of the free portion of the tongue, the fibers are predominantly longitudinally disposed, and then they are predominantly oblique, with a cranio-dorsal / ventro-caudal orientation. Among them, in the central area appear transversely arranged cells that are quite well represented, but decrease numerically to the boundary between the free portion and the body of the tongue. In the body of the tongue, the superficial dorsal layer is present up to the intermolecular protuberance. From the molar protuberance level and at the level of the tongue root, the superficial dorsal layer is no longer present. In this layer, the cells are predominantly longitudinal but numerically they become fewer towards the intermolecular protuberances, and with the numerical reduction of the longitudinally arranged cells, some vertical and horizontal oriented cells appear.

Regarding the orientation of cells in the ventral superficial layer, unlike the dorsal surface, they are predominantly longitudinally arranged, with scattered vertical or horizontal myofibers. Numerically, longitudinally oriented cells are dominant. Towards the intramolecular protuberance and at its level, the ventral superficial layer is formed by two cellular planes. One plane is ventrally located, in which the cells are located approximately parallel to the longitudinal axis of the tongue. The other plan is located dorsally and the cells have an oblique orientation. Surface-oriented superficial muscle layers are responsible to curve the organ dorsally and ventraly. The characteristic layout of muscle cells at this level and the fact that the two layers are well represented (especially the lower one) makes these movements to be carried out with force and precision.

In the area between the two superficial planes, the ratio of the cells placed vertically and horizontally is not identical across the free tongue. Thus, at the tip of the tongue, the orientation of the muscle cells is plexiform, then the cells in a vertical orientation predominate. According to this, in the middle third of the tongue, the ratio is approximately equal. In the last third, the cells with a horizontal layout predominate. In the posterior third of the free portion of the tongue, small lobules of adipocytes are present among myofiber bundles. At the body of the tongue, the central area is occupied by vertical and horizontal cells. The ratio between them is approximately equal. The well-ordered arrangement of myofibers facilitates a great horizontal and vertical mobility of the tongue in Chinchilla.

The ratio of the muscle component and the interfascicular conjunctive tissue is not identical across the entire sectional area. On the largest surface, muscle tissue is predominant, but at the intermolecular protuberance, the presences of the adipose lobules cause the connective tissue to occupy about half of the sectional area. Such infiltrated conjunctive tissue with adipocytes is also present in the dorsal half of the posterior portion of the intermolecular protuberance. At the level of the root (i.e., in the dorsal half), serous and mucous acini can be detected, which make the muscular tissue to be less dominant.

Longitudinal, vertical and horizontal muscular cells in the tongue have been described in mice (Yoshioka et al., 1979), rat (Bailey et al.2006; Abayomi et al., 2009; Ghassemi and Cheshmi, 2014), pangolins (Abayomi et al., 2009) hedgehogs (Goodarzi and Azarhoosh, 2016), bats (Abayomi et al., 2009).

Some authors state that in mice the muscular cells in the superficial dorsal layer are located longitudinally throughout the tongue length (Yoshioka et al., 1979). The results obtained by us in chinchilla differ quite a lot from those described in mice (regarding the pattern of the

superficial dorsal plane). We have found that in Chinchilla this layer is not present across the length of the tongue, and the cells are not only longitudinally available, but there were also differences from one segment to another. Thus, at the tip of the tongue, the arrangement of the muscle cells in the dorsal superficial layer is plexiform, gradually forming a thin layer with predominantly longitudinal orientation. Afterward, it retains the orientation and the layer gradually thickens. Regarding the ventral superficial layer, in Chinchilla, it extends from the intermolar projection to the tip of the tongue, so that both the extension and the orientation of the myofibers are similar to those described in mouse (Yoshioka et al., 1979).

Regarding the presence of glandular acini at the root level of the tongue, the aspect is supported by all the consulted authors, but there are also differences in some species. Many authors have reported two types of acini, serous and mucous, in a half from the dorsal root of the tongue in some mammalian species, such as the Iraqi Goat (Jabbar, 2014), the rat (Ghassemi and Cheshmi, 2014). In a similar way, the results obtained by us show that both types of acini are present in the tongue of Chinchilla. However, there are authors who claim that there are small acini in the rat, besides the serous and mucous ones (Ghassemi and Cheshmi, 2014).

Conclusions

In Chinchilla's tongue, the myofibers are oriented longitudinally, transversally, vertically and occasionally in an oblique way. The myofiber bundles are arranged in a superficial dorsal layer, a ventral layer, while the central axis of the organ occupies the space between the two superficial layers. The superficial dorsal layer extends from the tip of the tongue to the intermolecular protuberance. It is thin and plexiform on most of the free portion of the organ but gradually thickens towards the body of the tongue where the cells are predominantly longitudinally and partially oblique. The ventral superficial layer is relatively thin in the free portion of the tongue, consisting mainly longitudinal and scattered vertical or horizontal cells. In the body region of the tongue, the ventral superficial layer is thicker and arranged in two oblique layers. The central axis of the tongue includes myofibers with a vertical and horizontal orientation. The specific layout of myofiber bundles in Chinchilla's tongue could be related to the specific way of food prehension, mastication and oral transport of feed in this species.

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Study on luteal tissue treatment in angus cows with natural and synthetic analogue of prostaglandins

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Abstract

The objective of this study was to evaluate the effects of prostaglandins (natural and synthetic analogues) on estrous synchronization in beef cows. The study was carried out between May – August 2019 in a private unit in Timis County, and involved 54 Aberdeen Angus cows diagnosed with ovarian luteal tissue: corpus luteum (n=36 cows), cystic corpus luteum (n= 8 cows) and luteal cysts (n=10 cows). Two products were used for the treatment of this pathology: PGF Veyx Forte (n=27 cows) and Enzaprost (n=27 cows). The interval between hormonal treatment and the onset of estrous was ranging from 48-96 hours. In this trial 83.33% of the animals expressed signs of estrous. In the present experiment the conception rate after the artificial insemination was 71.11%.

Keywords: *beef, corpus luteum, prostaglandin*

Introduction

In no other species of farm animal is reproductive efficiency more important than in the cow. Gestation length is relatively long in the cow (283 days) and so any loss of production time is amplified many times over. At best, a cow can produce only one calf per year. The use of AI in combination with effective synchronization protocols can reduce the number of days open and accelerate genetic progress [7].

Reproductive management programmes based on strategic use of prostaglandin F2 alpha (PGF2 alpha) to induce and synchronize oestrus in cows are widespread. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin analogues (PGF) are luteolytic in cattle and other domestic species and usually induce oestrus when given during the luteal phase of the oestrous cycle. The interval between administration of the hormone and onset of oestrus ranges from 2 to 6 days [1, 9].

Three important factors regarding PGF as a treatment for estrus synchronization must be considered. Firstly, it is necessary that a functional corpus luteum (CL) be present for PGF to be effective. Early postpartum anestrous cows and prepuberal heifers are not good candidates for PGF protocols because they will not have a functional CL. Secondly, the CL prior to Day 5 after ovulation is nonresponsive to a single injection of PGF [6, 11]. The third factor to take into account when using PGF, is the great variability in the interval from treatment to behavioral estrus and ovulation among treated animals. In the presence of a responsive CL, estrus can be induced by a single administration of PGF; however, the interval to the resulting estrus and ovulation is dependent on the stage of development of the dominant follicle at the time of treatment [4, 5].

Estrous synchronization gives many beef cattle producers the opportunity to capture the economic benefits of artificial insemination (AI). Because AI involves a substantial investment of labor and time, most commercial farms or ranches will not utilize this technology unless this investment can be confined to a period of less than 5 to 7 days. To make the labor requirements of AI compatible with modern beef cattle breeding, the estrous cycle must be synchronized so that a high percentage of treated females show a fertile, closely synchronized estrus.

Compared with rectal palpation, ultrasound examination has been shown to be a more sensitive and specific method: 95% sensitivity and 100% specificity vs 95% and 95.7%, respectively, for rectal palpation [8]; 84% sensitivity and 71% specificity vs 79% and 40%, respectively, for rectal palpation [2].

Material and methods

The study was carried out between May – August 2019 in a private unit in Timis County, and involved 93 Aberdeen Angus older than 24 months.

After performing the rectal palpation (RP) and the ultrasound examination of the bovine female genital tract several ovarian luteal tissue disorders were observed: corpus luteum (n=36 cows), cystic corpus luteum (n= 8 cows) and luteal cysts (n=10 cows).

Luteal cysts formed a thicker wall of luteal tissue around their outer edges. Luteal cysts are often larger than normal corpus luteum.

A major advantage of ultrasound when dealing with these cysts is its ability to distinguish a luteal cyst from that of a very young corpus luteum (day 5 or 6 of the estrous cycle). An early corpus luteum also have a fluid-filled lumen and cobwebs as it continues to luteinize. Using rectal palpation, it is extremely difficult to palpate an early corpus luteum let alone distinguish it from a luteal cyst as they both have similar palpable features, but can be more clearly differentiated with an ultrasound.

Since luteal cysts invariably have luteal tissue and produce progesterone, the best treatment for resolving them is administration of prostaglandin F2α to initiate luteolysis.

All animals were divided in 2 equal groups (n=27 cows): 18 cows with CL, 4 cows with cystic corpus luteum and 5 cows diagnosed with luteal cysts.

The treatment in group 1 was performed with an synthetic analogues of prostaglandins – Cloprostenol (PGF VEYX FORTE, Intervet; Netherlands) and for group 2, a natural prostaglandin was chosen – Dinoprost (ENZAPROST). One ml solution of PGF Veyx Forte contains 0.263 mg Cloprostenol. A single intramuscular administration was performed in all cows in group 1 and the dose was 0.5 mg / animal (2 ml / animal). The cows from group 2 received 5 ml Dinoprost/animal (25 mg Dinoprost) in unique dose.

After the treatment the following parameters were investigated: estrous onset, duration and intensity of estrous, pregnancy rate.

Results and discussions

All the investigations in this trial were performed at cows that were more than 60 days after calving. At the ultrasound examination the luteal cyst presented the wall thicker, with a size larger than 4 mm. Regarding the cystic corpus luteum the diagnosis was set when at the ultrasound examination was found that the cavity of the corpus luteum was bigger than 1 cm.

After the hormonal treatment the animals were monitored until first clinical signs of estrus were detected.

The animals in group 1 expressed more intense signs of estrus than those in group 2: most females allowed other animals to mount them, the vulva was swollen and reddish, the area around the tail was wet and dirty, clear mucus from the vulva.

The onset of synchronized estrous was calculated from the time of administration of hormone to the time of first appearance of estrous symptoms. In group 1 the mean interval between cloprostenol treatment and the onset of estrous was 55.72 hours ranging from 48-96 hours. In this group, 5 cows did not express signs of estrous (Table 1). Regarding group 2 the mean interval between dinoprost treatment and the onset of estrous was 56.87 hours ranging from 48-97 hours.

In this group, 4 cows did not express signs of estrus (Table 1). Similar results in beef cattle were recorded (54.2 hours) [10].

The average duration of estrous in group 1 was 22.45 hours with limits between 18-36 hours and in group 1 the mean duration of estrous was 22.21 hours with individual values between 19-35 hours.

In our study the proportions of cows detected in estrus was 81.48% in group 1 and 85.18%in group 1. A study from 2002 reported values of estrous response between 44-100% depending on the dose of Cloprostenol used [3].

The cows detected in estrous were artificially inseminated.

After 32 days the gestation confirmation was performed. A total of 32 pregnant cows (71.11%) were diagnosed after the first gestation examination. In group 1 16 cows were pregnant (72.72%) and in group 2 a number of 16 cows (69.56%) were positives after performing the pregnancy exam (table 1). At the second pregnancy exam 9 cows were diagnosed with 1st degree ovarian hypotrophy and in 4 cows chronic endometritis was found.

Table 1. Fertility parameters recorded

Parameters	Values	
	Group 1	Group 2
Estrus response rate (%)	81.48 (22/27)	85.18 (23/27)
Onset of estrus (hours)	55.72	56.87
Duration of estrus (hours)	18-36	19-35
Conception rate (%)	72.72	69.56

Conclusions

It is possible to conclude that the treatment of ovarian luteal tissue disorders with prostaglandins (natural and synthetic analogue) is an efficient method for estrous synchronization in beef cattle.

The type of corpus luteum or the pathology of luteal tissue did not influence the pregnancy rates of bovine.

The overstimulation of the mammary gland by the calves leads to the transformation of the ovary with a corpus luteum into 1st degree ovarian hypotrophy.

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Study concerning the prevalence of ovarian diseases in Aberdeen angus cows

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Abstract
Infertility in cows is one of the major problems with great economic implications. The purpose of this study was to identify by global and individual gynecological investigation major ovarian diseases and to *determinate their incidence in the relation with hot season at Aberdeen Angus cows. The study was carried out in June 2019 in private unit in Timis county, Romania, on a number of 93 Aberdeen Angus cows in order to determine the prevalence of different ovarian diseases at this breed in a summer season. Gynecological investigations were performed in order to establish the prevalence of ovarian diseases in the summer season. The results showed different pathological entity variation. Thus, 50.22% of the animals taken into study presented ovarian hypofunction, 27.9 % presented corpus luteum , 0.93% polycystic ovary with uterine adherences and 7.44% ovarian follicle.*
Keywords: *infertility, gynecological investigations, beef cows.*

Introduction

The energy intake from feed cannot keep up with that required for milk production, causing a negative energy balance (NEB), especially due to a rapid increase in the milk yield after delivery. This is the reason for the recent reduction in the reproductive performance of dairy cows [6]. As a broad concept, fertility in a female mammal is defined as the ability to conceive and maintain pregnancy producing viable offspring. This requires regular cyclicity showing oestrus, ovulation of a healthy oocyte and an adequate environment in the oviduct and uterus for gamete transport and conceptus development [12]. Moreover, in warm countries, summer heat stress is a major factor affecting fertility in high-producing dairy and beef herds [3,7].

Thermal stress during the 3 days preceding insemination has been associated with decreased conception rates [4]. A cow was considered to suffer follicular anovulation or ovarian hypofunction when a follicular structure of at least 8–15 mm was detected in two consecutive examinations, in the absence of a corpus luteum or cyst and no oestrus signs were noted during the 7-day period between the examinations. Ovarian hypofunction disorder is characterized by the growth of the dominant follicle until 8–15 mm and the absence of ovulation [8]. Cystic ovarian disease is one of the most common reproductive disorders in dairy cattle and a major cause of sub-fertility [11].

Cows with a follicular structure larger than 20 mm in diameter (McNutt 1927) detected in either or both ovaries in two ultrasonographic examinations performed at 7-day intervals and in the absence of a corpus luteum and uterine tone were recorded as cows suffering from cystic ovarian disease [12]. The corpus luteum (CL) is a transient reproductive gland that produces progesterone (P), required for the establishment and maintenance of pregnancy. Although the regulation of bovine luteal function has been studied for several decades, many of the regulatory mechanisms involved are incompletely understood [10]. Conditions that are included in luteal dysfunction include inadequate P4 production, premature luteolysis and persistent CL. All these conditions

relate to a failure of the CL to secrete P4 in sufficient quantity to maintain the appropriate period of a luteal phase in an estrous cycle or pregnancy. Persistent CL have been reported in cows which showed loss of the conceptus, inflammatory conditions of the uterus or congenital anomalies of the uterine horns. Early embryonic death and abortion or fetal mummification have also been associated with persistent CL. Conditions associated with persistent Cl include endometritis (11.28) or pyometra; the inflammatory lesions disrupt the normal luteolytic function of the bovine uterus and allow a longer lifespan for the CL .

Uterus unicornis, a congenital anomaly due to failure of a uterine horn to form, has a similar mechanism causing persistent CL. Corpora lutea formed on the ovary ipsilateral to the missing uterine horn will persist longer than normal due to a lack of a luteolytic signal to the ovary from the missing uterine horn [3].

The purpose of this study was to identify by global and individual gynecological investigation major ovarian diseases and to determinate their incidence in the relation with hot season at Aberdeen Angus cows.

Materials and method

The study was carried out in June 2019 in private unit in Timis county, Romania, on a number of 93 Aberdeen Angus cows in order to determine the prevalence of different ovarian diseases at this breed in a summer season. Gynaecological investigations were performed with the ultrasound Easy Scan Linear in Ovary Mode. The animals were followed in order to determinate the formations from the ovary. Pathological and physiological structures were found, which we can list here: ovarian hypo function (Fig.1), corpus luteum (Fig.2), ovarian follicle (Fig.3) and polycystic ovary(Fig.4).

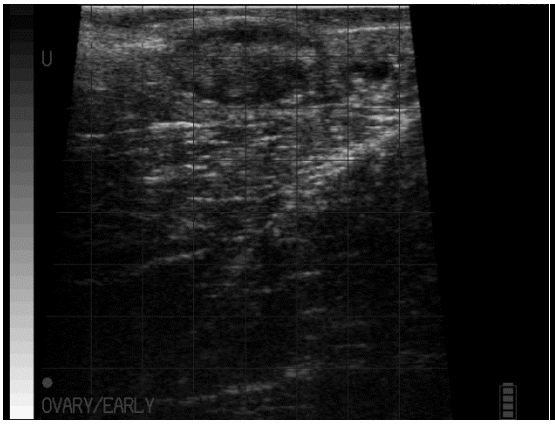

Fig.1 Ovarian Hypofunction

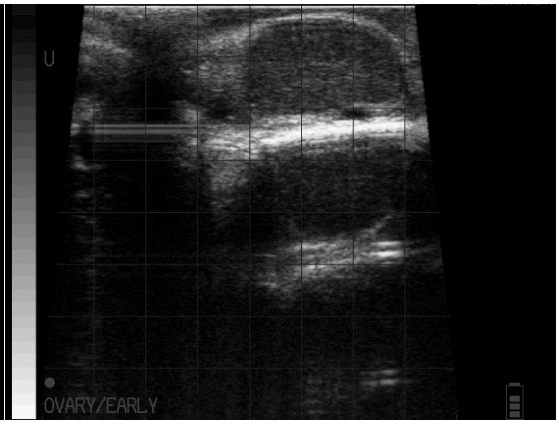

Fig. 2 Corpus Luteum

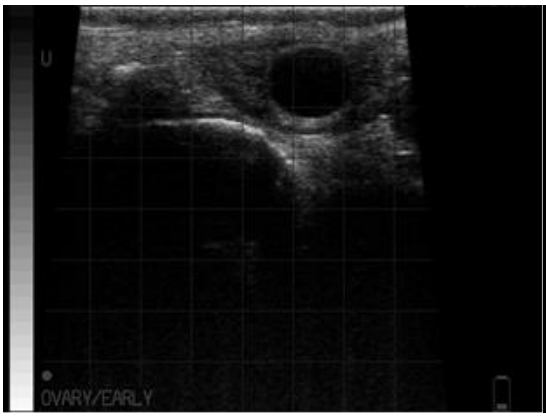

Fig. 3 Ovarian follicle

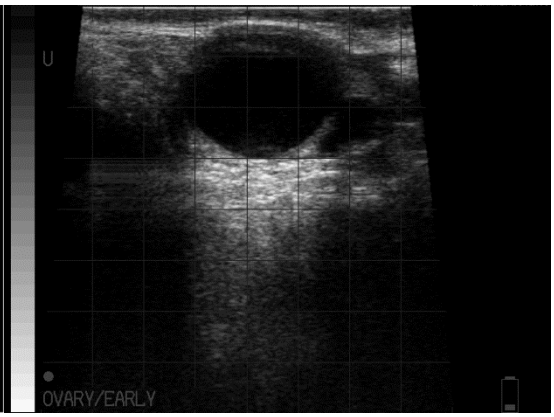

Fig.4 Polycystic ovary

Results and discussions

The results showed different pathological entity variation. Thus, 50.22% of the animals taken into study presented ovarian hypofunction, 27.9 % presented corpus luteum, 0.93% polycystic ovary with uterine adherences and 7.44% ovarian follicle (Tab. 1).

Tab.1 Incidence of the ovarium formations

Ovarian formations	Number of cases	Percent
Corpus luteum	30	28.9 %
Ovarian Hypofunction	54	50.22 %
Ovarian follicle	8	7.44 %
Polycystic ovary	1	0.93 %

The analysis of the recorded data within this study indicates a variation of the different types of anoestrus in relation with the type of ovarian formations. The ovarian hypofunction is the principal cause of the subfertility and the most common patology in the summer period at Aberdeen Angus breed. In our research the incidence of ovarian hypofunction was 50.22%, Herry Agoes Hermadi et all.(2017) reported an incidence of ovarium hypofunction in limits between 2.8%-4.7% and Yanzi et all. [12] reported ovarium hypofunction in limits between 3.8% and 12%. Ovarian hypofunction may be associated with intermediate LH pulse frequencies (1 pulse ⁄ 1–2 h) caused by low progesterone concentrations or by different sources of stress, such as heat stress [2], in our study the temperature in the study time was at the high values, values with limits between 28-35 °C. The incidence of ovarian chists was 0.93%, similar values were reported in literature, 2.8% and 0.7-1.24% [2,9]. Ioana Crivei reported in 2015 27.84% animals with ovarian diseases, of which 13.53% are represented by persistent corpus luteum, in our study the percent of corpus luteum incidence was 28.9%.The most commonly recognized causes of a persistent corpus luteum are ovulations late in diestrus, embryonic loss after the time of maternal recognition of pregnancy, and

chronic uterine infections (i.e., pyometra). Insertion of a sterile glass ball (e.g., a marble) into the uterus to prevent estrus, administration of oxytocin during mid-diestrus, and ovulation during a course of altrenogest administration can also cause persistence of the corpus luteum [11].

Conclusions

The ovarium hypofunction is the most commonly pathology at the Aberdeen Angus Breed in the summer season. The heat stress is one of the principal causes of ovarian hypofunction.

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The treatment of retained placenta with an intrauterin suspension

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Abstract
The purpose of this research was to evaluate an intrauterine suspension in the treatment of retained placenta. A therapeutic option that affects, as little as possible the female reproductive life, thus reducing the associated economic implication is the ideal in the treatment of retained placenta. In this study, we analyzed the cases of retained placenta in a farm from Cluj county, the incidence, the diagnosis and the treatment of this pathology in cows. The study was carried out during January 2017-April 2019 and included 216 animals that have calved in that period. Of these, 16 animals were with retained placenta at 12-15 hours after calving, which represents 7.40%. After the treatment, the service period, the number of doses of semen needed for one gestation and the costs of treatment were established.
Keywords: *retained placenta, treatment, intrauterine suspension*

Introduction

Retained placenta is a frequently diagnosed uterine disease in early-postpartum cattle, witch has several different etiologies, including abortion, dystocia, hypocalcemia, twin birth, induction of parturition, placentitis, nutritional disturbance and immunosuppression; thus, retained placenta remains therapeutic challenge in cattle, and alternative options should be considered [8]. Reproductive performance is linked to health in the weeks immediately before and after calving, and timely achievement of subsequent pregnancy in turn has a substantial impact on profitability [16]. Retention of fetal membranes in cattle can lead to adverse health effects that ultimately affect reproductive performance. The definition of retained fetal membranes is varied, ranging from retention of the placenta for 81 to 48 hours postpartum. Most studies define retained placenta in cattle at 12 to 24 hours, and therapy is usually instigated during this time. The majority of cattle (66% in one study) will pass the placenta within 6 hours after parturition [16]. Generally, the dominant approach to retained placenta in cattle in the field is to locally or systemically administer antibiotics. Some authors demonstrated that intrauterine antibiotic treatment is beneficial for metritis prevention in cows affected with retained placenta [8, 14]. Current reports indicate that antibiotic therapy, including intrauterine antibiotics and systemic antibiotics, generally has low efficacy in hastening the separation and expulsion of the retrained placenta [12]. Also, some authors reported that ozonated foam administered into the uterus is useful in treating retained placenta in cows resulting in beneficial effect on puerperal health and fertility [13, 1]. Collagenase therapy is one candidate approach, although it is considered too costly for widespread use [5]. Manual removal of the placenta still remains a common practice despite numerous studies that fail to demonstrate a beneficial effect on reproductive performance or milk yield, manual removal can result in more frequent and severe uterine infections, when compared with more conservative treatment [2]. The most commonly used hormone products in treating retained placenta are prostaglandins and oxytocin. These hormones play a role in uterine contraction, and could be effective in treating this pathology because of uterine atony. However, it is thought that uterine atony accounts for a very small percentage of retained placenta cases [10]. Because retained

placenta negatively affect milk production and cow's fertility, effective treatment is crucial for improving puerperal performance of cows in order to raise their productiveness [15].

The purpose of this research was to evaluate an intrauterine suspension in the treatment of retained placenta.

Materials and method

The study was carried out during January 2017-April 2019 on 216 cows that have calving in that period, in a dairy farm from Cluj County. All the animals were followed after the parturition and if the placenta was not eliminated at 12 hours after the parturition the diagnosis of retained placenta was set. The treatment of retained placenta was performed with an intrauterine suspension (Puerperal) made at the Faculty of Veterinary Medicine of Cluj Napoca. The product Puerperal has in composition: betadine, lincospectine, vitamins, boric acid and kaolin. All the components are important for the health of the uterus and also for rebalance the uterus medium. At all the animals was administered intrauterine 150 ml of Puerperal when the diagnosis of retained placenta was set, after that, were made another 2 administrations at 48 hours. Before intrauterine administration of the product, it is necessary to have a uterine massage to remove some of the content. The cows were monitored for established the service period, the number of doses of seminal material needed for 1 gestation and the costs of treatment.

Results and discussions

The placental retention diagnosis in cows under study was set at 12 to 15 hours post-partum, thus taking the decision to institute the therapeutic protocol. From 216 cows that were monitored a number of 16 (7.40%) animals has been found with retained placenta, 7.78% in 2017, 5.81% in 2018 and 10% in 2019. The situation in each year is presented in Figure 1.

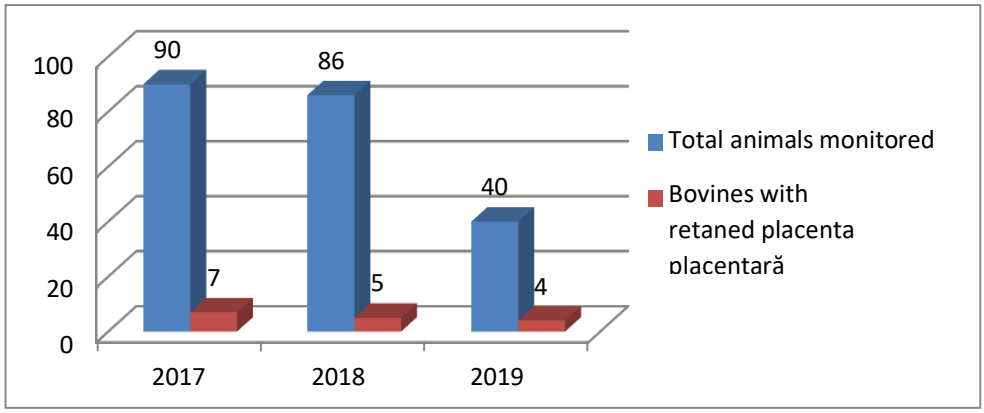

Figure 1. Situation in each year

From the animals with post-partum infection 81.25% were recuperated and 18.75% were out from the study because developed some postpartum infections (Fig.2).

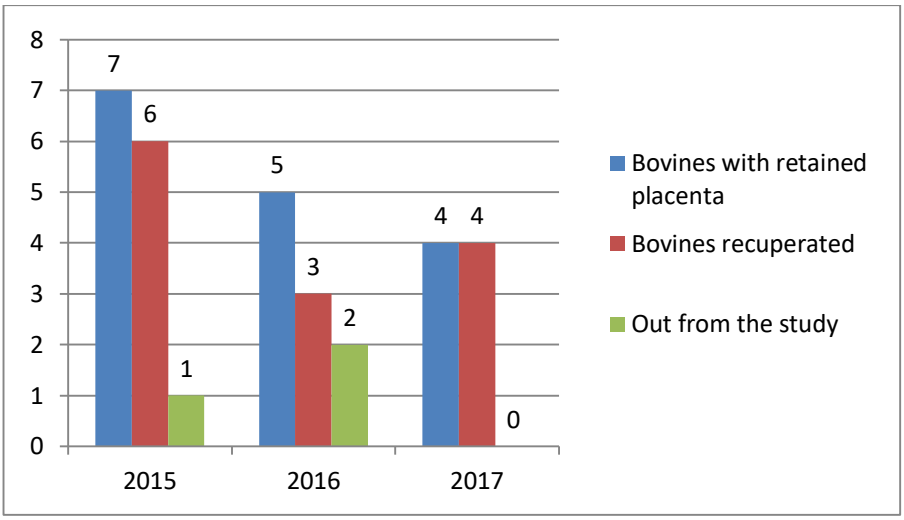

Figure 2. Situation after the treatment

The average of artificial inseminations at the animals with retained placenta was 1.84, in 6 cases was necessary only 1 insemination for obtain a gestation, in 3 cases were necessary 2 inseminations and in 4 cases 3 inseminations (Figure 3).

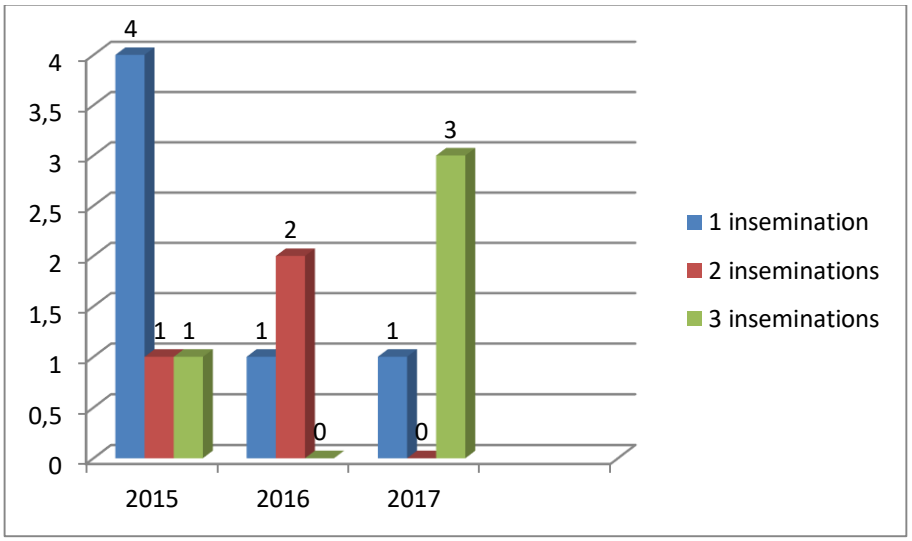

Figure 3. Number of artificial inseminations necessary for 1 gestation in each year

The service period has values in interval between 55 and 129 days with an average of 90.19 days. The situation for all the animals in each year is presented in Figure 4.

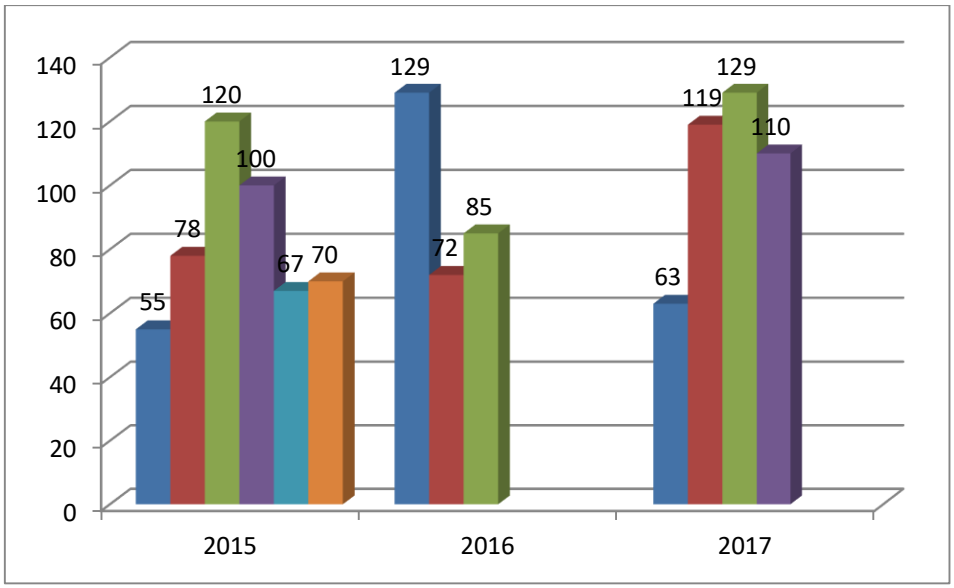

Figure 4 Service period for each cow

The average of cost for 1 animal with retained placenta was 180 RON, cost that included the consultation, the product and the administration of this one. For all the animals the total cost was 2880. For cows treated by a veterinarian, a cost of $244 per affected cow was estimated [7]. Kossaibati and Esslemont [9] calculated the direct cost of a case of retained placenta to be about £ 83, with an over-all cost of £ 298.29, in our study the value of cost was lower by we calculated just the costs with the treatment or the relative economic impact in retained placenta included: decreased milk production (40%), increased veterinary services (32%), increased culling rate (19%), and increased calving interval (9%).

The incidence of retained placenta varies from 4-18% of calving [11]. However, it can be much higher in problem herds and also increases during summer with increased parity, milk yield in the previous seasons and following birth of male fetus [3] Abortions, stillbirths and twin calving resulted in increased incidence rates of 25.9, 16.4 and 43.8%, respectively. In our study, the incidence was 7.40, with the biggest value in the year 2019 when the animals were monitored just in the first part of the year. Irina Garcia-Ispierto and Lopez-Gatius [4] reported 2.7 ± 1.7 artificial insemination for 1 gestation (1–9 inseminations), respectively (mean ± SD, range in parentheses). In our study were necessary 1.84 doses, we put these good results on the fact that we worked on small farms where it is easier to monitor cases. Han and Kim [6] reported a service interval of 83-85 days for the cows with retained placenta, in our research the average of service period was 90.19 days with values between 55 to 129 days.

Conclusions

After the treatment with Puerperal the average number of artificial insemination needed for 1 gestation was 1.84 and the average number of service period 90.19 days. The cost of the treatment with Puerperal for 1 cattle is 180 RON. The results of the study have shown the role of the product in regulating the physiology of the uterus and in increasing the conception rate.

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Estrous synchronisation and artificial insemination in out of breeding season at lacaune sheep

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Abstract
The aim of this study was to increase fertility in sheep by applying modern biotechnology: induction and synchronization of estrous in out of breeding season. The study was conducted during May 2019- July 2019 on 132 ewes, aged between 1 and 6 years, in Covasna county, Romania. The ewes were syncronisated with Medroxyprogesterone (Ovigest, Hypra), Cloprostenol (PGF Veyx Forte, Esteve) and GnRH (Ovarelin, Ceva). After the synchronization, the artificial insemination was performed at 126 ewes by intracervical method. At 29 days after the artificial insemination was performed the diagnosis of gestation using an ultrasound. From 126 animals artificial inseminated, in 121 (91.67%) cases the diagnosis of gestation confirmed the gestation and in 22 cases was confirmed a twin gestation.
Keywords*: estrous, sheep, synchronization.*

Introduction

Sheep is seasonal anestrous, which is controlled by photoperiod, whereas; nutritional and environmental cues restrict the potentiality of attaining the global target of three lambing per ewe every two years [12]. Ewes in seasonal anestrous have lower circulating levels of LH, FSH, and estradiol with basal levels of progesterone [4]. A distinct decrease in the LH pulse frequency is the hallmark endocrine feature of ewes during seasonal anestrous which is imputed to two major inhibitory mechanisms: (1) the hypothalamus is highly sensitive to the inhibitory effects of estradiol on LH release, even at low concentrations and (2) a direct inhibitory effect of long photoperiod on the hypothalamic–pituitary–adrenal axis [6]. Estrous synchronization of sheep has been accomplished using several methods with varying degrees of success [5]. Among the different methods used are the administrations of hormones like eCG, progestogens, prostaglandins either alone or in combination. Administration of progesterone/progestogens prevents the ewe from returning to estrous and ovulating and, therefore, supplementing the ewe with progesterone for a period equal to the duration of the life of corpus luteum (CL) and then withdrawing it will synchronize the release of gonadotropins causing estrous and ovulation in groups of ewes [8]. Progestogen impregnated intravaginal sponges or implants, PGF2α alone or in combination with gonadotropins have been widely used for estrous synchronization in sheep both in and out of breeding season [1]. Saxena et al. (2015) reported a nonhormonal intervention that induced ovulation in seasonally anestrous ewes that provides proof of principle that fertile estrous can be induced in seasonally anestrous ewes using the dopamine antagonist such as sulpiride [12].

Fixed-time artificial insemination (FTAI) is an important reproductive tool in animal production system. It has a direct impact on cost-efficiency by saving time and labor for estrous detection. However, if this package is able to produce acceptable estrous synchronization and lambing percentage during non-breeding season in the field conditions, then this technology can be used for accelerated lamb production and three lambs in two year production system [7]. Whereas, the use of AI sheep declined and for this reason the farmers are more reticent towards

this technique, preferring the natural one. The advantage of this technique in sheep, is not only limited to obtain good results with the control cycles technique, but additionally facilitates the establishment of selection schemes in the offspring [10].

The aim of this study was to increase fertility in sheep by applying modern biotechnology: induction and synchronization of estrous in out of breeding season.

Matherials and method

The study was carried out during May 2018 - July 2019 in a sheep flock from Covasna county Romania (lat. 44.4267674, long. 26.102538390000063). A number of 132 ewes from Lacaune breed with age between 1-6 years were synchronized with intravaginal sponges with Medroxyprogesterone (Ovigest, Hypra), Cloprostenol (PGF Veyx Forte, Esteve,) and GnRH (Ovarelin, CEVA). After that, the ewes were artificial inseminated by intracervical method at fixed time.

The sponges (60 mg Medroxyprogesterone) were inserted and kept in situ in the vagina for a period of 12 days. 50µg of GnRH and 250 µg of Cloprostenol were administered intramuscularly at the time of sponge withdrawal on 12^{th} day. Cervical insemination was performed in ewes exhibiting estrous (restlessness, shaking of tail, slightly swollen vulva, moist and reddish cervical external os) at 53-55 hours after the removal of sponges. For artificial insemination was used fresh semen collected from 3 rams witch had higher body weight and produced a high quality sperm. The semen was collected by artificial vagina method, after that, a 1/1 dilution of the seminal material was performed. After collection, the semen samples were evaluated for volume, consistency, wave motion (0–5 scale), density and % motile spermatozoa (0–100 %). After evaluation, a second dilution of the semen samples was performed, finally the samples were 1/10 diluted. The artificial insemination was performed with 0.25 ml of semen by intracervical method. At 29 days after the insemination the ewes were diagnosed for gestation by transrectal method with a longitudinale sonde using the frequency of 5 mH.

Results and discussions

Estrous synchronization in out of season was performed in a farm from Covasna county, Romania at 132 ewes. In the first day of the experiment 132 sponges with Medroxyprogesterone were intravaginal introduced. After 12 days a number of 131(99.24%) sponges were collected, 0.76% sponges had fallen out during this period. All the ewes were intramuscularly injected with Cloprostenol and GnRH in day 12, at 53-55 hours after the administration, 126 (95.45%) cases showed estrous signs, so, this animals were artificial inseminated by intracervical method. From 126 animals inseminated in 121 (96.03%) cases the diagnosis of gestation was positive and 3.96% the diagnosis of gestation was negative. In 22 (18.18%) cases the diagnosis of twin gestation was set (Table 1).

Table 1 Estrous synchronization and fixed-time artificial insemination in out of breeding season at Lacaune sheep under field conditions of Covasna county (Romania)

Indicator	Number (percent)
Total animals	132 (100)
Sponge in	132 (100)
Sponge out	131 (99.24)
Ewes in heat	126 (95.45)
A.I ewes	126 (95.45)

Gestations of total ewes	121 (91.66)
Gestations of A.I ewes	121 (96.03)
Number of twin gestations	22 (18.18)

The percent of sponge out after the day 12 of synchronization was 98.04%, higher than in a study of 2014 [9] where the number percent of sponge out was 96.07. Estrous activity including rate, onset and duration of estrous. In our study 95.45 % of ewes responded to treatment and exhibiting estrous signs. Nasser et al. (2012) found that estrous response of ewes treated with CIDR for 12 days + eCG or 6 days + eCG was 100% for both treatments [10]. However, Kasikci et al. (2011) reported that lower estrous response of Tahirova ewes treated with whole sponge (20 mg), halved sponge (10 mg), whole sponge + 600 IU eCG, whole sponge + 300 IU eCG, halved sponge + 600 IU eCG and halved sponge + 300 IU eCG, being 91, 96, 86, 96, 98 and 94% [9]. Özbey and Tatle [11] synchronized the Awassi ewes for 14 days with sponges containing 40 mg of FGA and superovulated by 500 IU of PMSG injection and estrous and twinning rates were 100% and 46%, in our study the twinning rate as 18.18%. The use of freshly diluted semen could give the best result: 70 to 82 % [2] and 82.2% [3] of pregnancy rate, in our study the pregnancy rate was 91.66%.

Conclusions

The results of estrous synchronization and artificial cervical insemination in out breeding season with fresh diluted semen were acceptable, 95.45 % ewes showed estrous signs, gestation percent was 91,66% with 22% of twin gestation.

Estrous synchronization and artificial insemination in out of breeding season can be performed with good results in Lacaune sheep in Romania.

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Studies regarding the clinical management optimization in babesiosis in dogs

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Abstract
The diagnosis of canine babesiosis is based, most of the times, on the patients' clinical signs, correlated with the ticks' parasitism, the emergence season, but the most important clue is performing the laboratory exams.The manifestations of the disease had, in most of the studied individuals, as a characteristic, the well-known clinical tetrad: fever, icterus, anemia and hemoglobinuria, mentioning that for the diagnosis confirmation it was necessary to perform the blood smear microscopic examination and to detect the intracellular parasites or the damaged erythrocytes.In the group of dogs with babesiosis, the haematological examination revealed a distinctly significant ($p<0.01$) decrease of the mean number of erythrocytes, indicating a severe haemolytic anemia caused by their massive destruction due to the parasites' mechanical and toxic actions, that along with the destruction of the immature erythrocytes (reticulocytes) - an indicator of regenerative erythropoiesis, ultimately causes tissue hypoxia. There is also found a statistically significant decrease ($p<0.05$) of the mean values of the haemoglobin and haematocrit. In dogs with babesiosis was noticed a statistically significant increase ($p<0.05$) of the urea's mean value and an increase (but not significant - $p>0.05$) of the creatinine's mean value, compared with those of the control group. Thus, a major role in the pathogenesis of the renal function impairment in dogs with babesiosis can be attributed to the septic-toxic state induced by the intraerythrocytic parasites. The mean value of the bilirubin in the group of dogs with babesiosis was distinctly significant higher ($p<0.01$) compared to the control group, a sign of massive erythrocytes' degradation, which stands at the basis of the haemolytic anemia diagnosis and thus the liver function decrease, translated by the significant increase ($p<0.05$) of the hepatocellular injury specific enzymes activity (ALT). Another biochemical change was hypoglycaemia. In this context, the association between severe anemia and hypoglycaemia may be explained by the effect of hypoxia on the glycolysis and by the fact that the severity of the anemia is closely related to the disease severity and the inflammatory response intensity, which may lead to an intensification of metabolic processes and, consequently, to an excessive glucose consumption.
Key words: *babesiosis, dogs, haematological and biochemical exams*

Introduction

Due to the two factors involved, the host organism (of different breeds and ages, at which different collateral conditions may or may not be present) and the parasite (of different species, three identified in Romania), babesiosis develops and expresses a highly complex and intricate clinical-pathogenically and paraclinical ensemble (*10*).

The purpose of this paper is to contribute to the identification and integrated assessment of the epidemiological framework and coordinates of babesiosis on an delimited area in Ilfov county and to evaluate and subsequently appreciate in an integrated manner the prognostic values of the variations of some haematological and biochemical parameters.

As mentioned in the speciality literature, the diagnosis of canine babesiosis is most often based on the clinical signs of the patients, correlated with tick parasitism, season of onset (*8,9,11*), but the most important clue is represented by the performing of the laboratory exams (*6,7*).

The haematological abnormalities observed during the acute form of the disease were also mentioned by other authors (*14*), who point out that the major mechanism of producing serious

imbalances in the body is ischemia, because a very large number of erythrocytes that are parasitized are retained and destroyed in the spleen.

In this context, the body cannot produce new erythrocytes at the rate at which they are destroyed and, thus, increases the number of reticulocytes - an indicator of regenerative erythropoiesis (*3,12,13*), which leads to irreversible changes at the liver, splenic or renal level (even with lungs or cardiac complications).

Regarding the symptoms of babesiosis in the acute form, they were generally uniform and translated, in most cases, by apathy, fatigue, anorexia, loss of appetite, febrile syndrome characterized by sudden hyperthermia (39.8-41ºC), anemia, jaundice, hemoglobinuria, respiratory disorders (*2,5*).

It is worth mentioning the fact that, in most cases, the manifestations of the disease had, as a characteristic, the well-known clinical tetrad, represented by fever, icterus, anemia, hemoglobinuria, also mentioning the fact that in order to confirm the diagnosis, the microscopic examination of a blood smear of peripheral blood and highlighting intracellular parasites or the damaged erythrocytes were necessary (*1*).

Materials and methods

The present study was performed on a number of 67 dogs belonging to several breeds, of different ages and genders, presented and consulted at the Konivet Medical Veterinary Clinic, Domnești commune, Ilfov county, but also at the clinic within the Faculty of Veterinary Medicine Bucharest, from 2016 until 2018.

It is worth mentioning the fact that, due to the quite large variations of the haematological and biochemical parameters that exist between different breeds of dogs, for the accuracy of the results and for a more accurate statistical interpretation, the patients in the control group were selected in such a manner that, they would largely belong to the same breeds as the patients with babesiosis.

The dogs in the group suspected of babesiosis, underwent rigorous physical and clinical examination. During the clinical examination, in the majority of the infested dogs (and included in the studied group), the presence of ticks could be observed on the skin.

Since the identification of endoglobular parasites in the blood smear represents the decisive element of the etiological diagnosis, the infested dogs were included in the studied group after at least two smears of peripheral blood (*May-Grumwald* coloured) were performed in order to detect the *Babesia spp.* parasites from the red blood cells.

In order to confirm the diagnosis and establish an individualized therapeutic protocol in patients with suspicion of babesiosis, it was preceded to harvesting biological samples (whole blood on EDTA) for haematological examination (to determine the degree of anemia) and biochemical exams (to determine the degree of impairment of the hepatic and renal functions).

Anamnestic data and disease history are an important component in the preparation and implementation of the protocol and the clinical examination chart, which is why the elements that are revealed from the (detailed) anamnesis may highlight various pre-existing or competitive conditions, as well as the clinical signs, but also the reason for presentation at the consult and medical evaluation.

The haemogram, which is a basic screening test, associated with examination of the blood smear, provide valuable information about the anemia degree and more. For the complete blood count, blood was harvested (by venous puncture) in special tubes with anticoagulant.

The automatic analyser used to perform the haematological determinations was Melet Schloesing MS 45, and for the determination of the biochemical parameters it was used the

Spotchem EZ 4430 ARKRAY analyser, a dry biochemistry analyser that uses as a principle for the parameters determination the optical measurement of the reflection intensity (*4*).

Results and discussion

Dogs infested with *Babesia spp.* present a clinical picture with various manifestations of the disease, the incubation period being comprised between 7 and 14 days, but depending on the pathological condition of the patient, the clinical signs of the disease can appear even from the 5th day of infestation.

The manifestations of the disease had in most of the studied individuals, as a characteristic, the well-known clinical tetrad, represented by fever, jaundice, anemia, hemoglobinuria, mentioning that for the confirmation of the diagnosis it was necessary the microscopic examination of a peripheral blood smear and to highlight the intracellular parasites in order to confirm the diagnosis. The clinical tetrad (fever, jaundice, anemia, hemoglobinuria), specific to canine babesiosis, is well represented also in the studied group. Thus, 84.2% (n=16) of dogs with babesiosis had a rectal temperature above 39,2^{0}C (Chart 1).

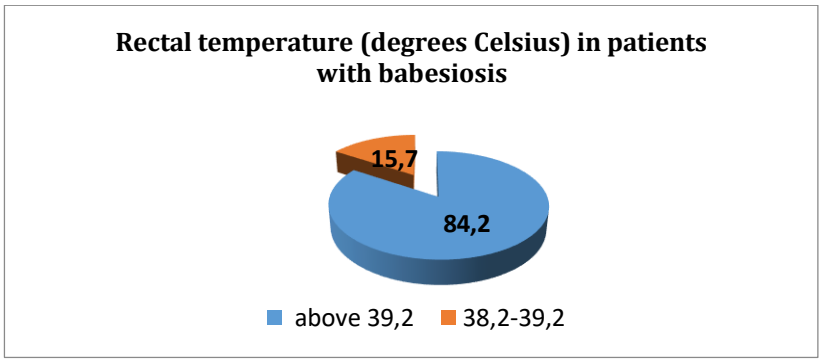


Chart 1. Representation of the percentage of dogs with babesiosis that presented fever

Also, it can be mentioned the fact that 47.3% of the dogs from the group with babesiosis presented at clinical examination jaundice at the level of the mucous membranes and/or the abdominal region skin, and more than half of them (57.8%) also presented hemoglobinuria (Chart 2). Noteworthy is the fact that a very large percentage of the dogs with babesiosis, of the studied group, (89.4%) presented paleness of the mucous membranes.

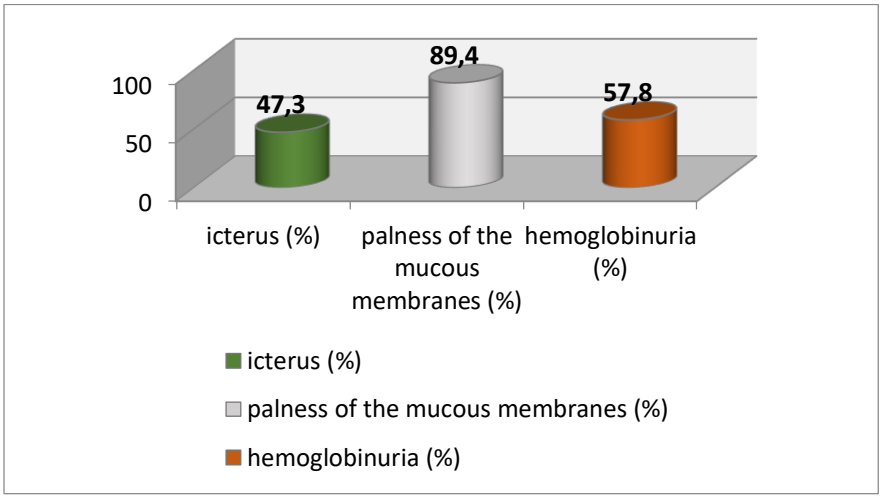


Chart 2. Representation of the percentage of dogs with babesiosis which presented different clinical signs

Following the comparative analysis of the haematological and biochemical parameters in dogs from the babesiosis group and of those from the control group, significant differences were observed, as other authors have also pointed out (*5*).

In this context (Table 1), in dogs from the group with babesiosis, the haematological examination revealed a distinctly significant decrease ($p<0.01$) of the average number of erythrocytes (4.4 ± 0.8 mil/mm^3), compared to the control group (6.45 ± 0.9 mil/mm^3), which denotes a severe haemolytic anemia caused by the massive destruction of the red blood cells following the mechanical and toxic action of the parasites, which ultimately causes tissue hypoxia due to the massive destruction of the red blood cells, but also of the immature erythrocytes (reticulocytes) - an indicator of regenerative erythropoiesis, which get into circulation under the action of erythropoietin (*14*).

In dogs from the babesiosis group (Table 1), was observed a statistically significant decrease ($p<0.05$) of the mean values of haemoglobin (9.1 ± 0.3 g/dl) and haematocrit (30.4 ± 6.4%), compared with the average values of these parameters in the control group (13.8 ± 1.0 g/dl, respectively, 39.7 ± 2.4%).

Table 1. The mean values of the haematological and biochemical parameters in patients with babesiosis

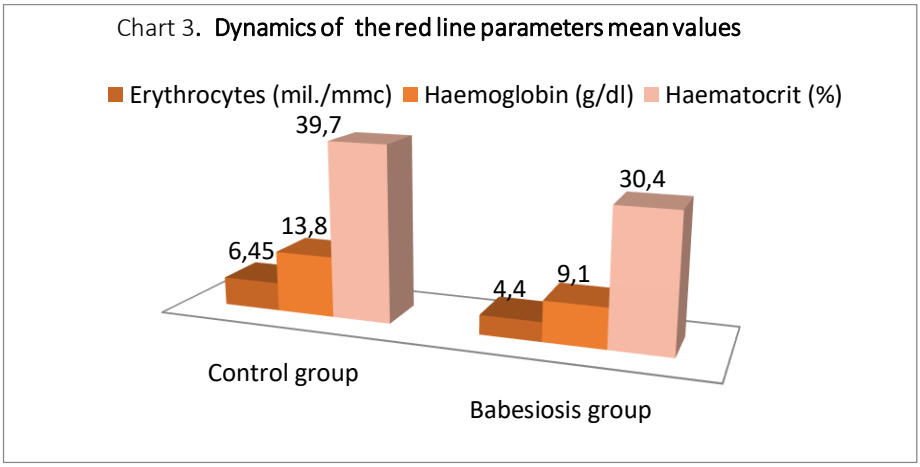
No.	PARAMETER	The mean values in the control group *(n=13)*	The mean values of the patients with babesiosis *(n=19)*	The limit values recorded in the patients with babesiosis *(n=19)*
1.	**Erythrocytes** *(mil/ mm^3)*	6.45 ± 0.9	4.4 ± 0.8***	3.8-5.0
2.	**Haemoglobin** *(g/dl)*	13.8 ± 1.0	9.1 ± 0.3**	8.5-9.7
3.	**Haematocrit** *(%)*	39.7 ± 2.4	30.4 ± 6.4**	26-34
4.	**Total leukocytes** *(mii/mm^3)*	9.2 ± 0.8	8.6 ± 0.4*	8.0-9.2

5.	**Leukocytes formula**	**Neutrophils** *(%)*	58 ± 7	41 ± 2**	37-45
6.		**Lymphocytes** *(%)*	30 ± 8	43 ± 2**	39-47
7.		**Monocytes** *(%)*	7 ± 4	10 ± 3*	7-13
8.	**Bilirubin** *(mg/dl)*		4.04 ± 0.2	3.94 ± 0.2***	0.8-11.2
9.	**Total proteins** *(g/dl)*		6.7 ± 0.21	5.4 ± 0.24*	4.9-5.9
10.	**Albumins** *(g/dl)*		3.1 ± 0.05	2.3 ± 0.08**	2.0-2.6
11.	**Globulins** *(g/dl)*		3.6 ± 0.2	3.1± 0.4*	2.7-3.4
12.	**A/G ratio**		0.86 ± 0.1	0.73 ± 0.10*	0.61-0.86
13.	**Glucose** *(mg/dl)*		119.6 ± 6.2	87.4 ± 5.0**	52.0-101.4
14.	**ALT** *(U/l)*		17.4 ± 1.5	51.0 ± 8.0**	37-65
15.	**Urea** *(mg/dl)*		15.6 ± 0.9	24.8 ± 2.2**	19-31
16.	**Creatinine** *(mg/dl)*		0.9 ± 0.06	1.4 ± 0.1*	0.8-2.0

** p>0,05 – no significant differences*
*** p<0,05 – significant differences*
**** p<0,01 – distinctly significant differences*

Worth mentioning is the fact that the clinical manifestations described in the group of dogs with babesiosis, together with the significant decreases in the parameters of the red line (Chart 3) - are characteristic of a severe haemolytic anemia, with the classic symptoms of intravascular erythrolysis being met: jaundice, paleness of the mucous membranes and fever.

The changes with statistical significance of the red line indicate the fact that the major mechanism of producing some serious imbalances in the body is ischemia, because a very large number of erythrocytes that are parasitized are retained and destroyed in the spleen.



In the studied group of dogs, there is found also a statistically significant increase (*p<0.05*) of the mean value of urea (24.8 ± 2.2 mg/dl), but also an increase (not significant - *p>0.05*) of the mean creatinine value (1.4 ± 0.1 mg/dl), compared with the mean values of the control group (15.6 ± 0.9 mg/dl, respectively 0.9 ± 0,06 mg/dl), this fact being the consequence of the septic-toxic state

induced by the parasites, in addition to the massive purge of haemoglobin and bilirubin, with direct toxic effect on the nephrons (Chart 4 and Table 1).

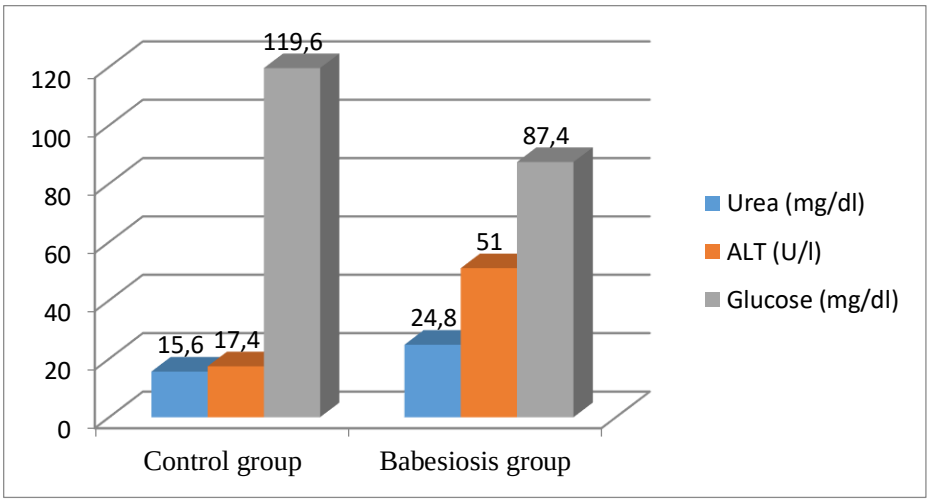


Chart 4. The mean values of urea, ALT and blood glucose in dogs from the control and babesiosis groups

Also, we notice the appearance of some statistically significant changes also in the case of the enzymes with specificity for hepatocellular lesions (Chart 4), with an increase ($p<0.05$) of the ALT activity in dogs with babesiosis (51.0 ± 8.0 U/I) compared to the mean value of the control group (17.4 ± 1.5 U/I).

Following the dosing of bilirubinaemia, it was found that the mean value of this parameter is distinctly significant higher ($p<0.01$) in the group of dogs with babesiosis (3.94 ± 0.2 mg/dl), compared to the mean value of the control group (0.3 ± 0.2 mg/dl), being a sign of the massive degradation of the erythrocytes that stands at the basis of the diagnosis of haemolytic anemia, having as a consequence the decrease of the hepatic function. Hypoglycaemia (87.4 ± 5.0 mg/dl) detected in patients with babesiosis can be attributed to and correlated with the effect that the hypoxia had on the glycolysis (Table 1).

Conclusions

1. In the studied group of dogs with babesiosis, the decreases (with statistical significance) of the red line parameters, corroborated with the clinical manifestations that constitute the specific tetrad of this hemosporidiosis - are characteristic of a severe haemolytic anemia, caused by the massive destruction of the erythrocytes consecutive the specific action (mechanical and toxic) of the parasites, and bring together the symptomatic assembly of intravascular erythrolysis: anemia, icterus, hemoglobinuria and fever.

2. The analysis of the complete blood count in dogs from the group with babesiosis, highlighted a distinctly significant decrease ($p<0.01$) of the average number of erythrocytes and also a statistically significant decrease ($p<0.05$) of the mean values of haemoglobin and haematocrit compared with the control group.

3. The clinical manifestations specific to the babesiosis had a general character, observing the fact that 47.3% of the patients had jaundice of the mucous membranes and / or of the hairless and depigmented skin, 57.8% had hemoglobinuria, 84.2% showed a hyperpyrexia background -

body temperature over 39.2^0C, and at a high percentage (89.4%) there was appreciated an obvious paleness of the apparent mucous membranes (correlated with the intensity of the haemolysis).

4. The results of the biochemical examination in the patients with babesiosis revealed the presence and intensity that can be correlated with the presence and the degree of toxic-septic states (induced by intraerythrocytic parasites), in the pathogenesis of the renal and / or hepatic function.

5. The biochemical parameters which attest the degree of functioning and the renal morphofunctional integrity undergo significant changes, evidenced by the statistically significant increase ($p<0.05$) of the mean value of the urea (24.8 ± 2.2 mg/dl), in conjunction with an increase ($p>0.05$) of the creatinine mean value (1.4 ± 0.1 mg/dl).

6. The distinctly significant increases ($p<0.01$) of the average values of bilirubinaemia (3.94 ± 0.2 mg/dl) in the individuals with babesiosis, compared to those registered in the control group, certify the dominant pathogenetic degradation of the red blood cells, constituting a preliminary and early diagnosis criteria of haemolytic anemia (including in the subclinical forms), with aggressive repercussions that have the effect of altering the liver function and a direct toxic effect on the nephrons, as a result of the massive clearance of haemoglobin and bilirubin at this level.

7. The identification of hypoglycaemia (87.4 ± 5.0 mg/dl) in patients with babesiosis can be attributed and correlated with the effect of the hypoxia on the glycolysis, a biochemical modification that can be associated with the degree and intensity of the anemia, jaundice and vascular collapse, in correlation with the rest of the lesions identified and associated with the inflammatory response, as well as the modifications/ alterations of the carbohydrate metabolism.

8. The blood cytomorphological examination (blood smear) in patients with babesiosis revealed a variable number of intraerythrocytic *Babesia spp*. parasites, (depending on the degree of impairment and the moment when the patient was brought at the veterinarian), constituting the element that allows the obtaining of a diagnosis of certainty (etiological) and identification, assessment and quantification of the morphological changes of the red blood cells (mainly anisocytosis and poikilocytosis), which undoubtedly attests the intense demand of the haematogenous marrow (in the process of erythropoiesis), indicating and attesting the regenerative character of the anemia.

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The importance of the bacterial cultures used in production of cheeses

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Abstract
The scientific researches reflected in this study has the purpose to study the microbiological aspects of the importance of bacterial cultures used in the production of the important dairy product-the cheeses. There have been studied some determinations of interactions between lactic acid bacteria, which are highly complex and beneficial for growth promotion activity of fermentation in the cheeses, and the use of selectioned cultures of lactic acid bacteria, which are used in the form of monocultures or mixed cultures with biotechnological suitable properties for the production of quality products in everyday life.
Key words: *Cheeses, Lactic microorganisms, Maia, Lactose, Microbial Enzymes.*

Introduction

Under the action of the selectioned cultures of microorganisms used as starter cultures in milk processing, over 600 possible variants are obtained in a wide range of assortments of cheeses. To obtain cheese, is used milk, in which lactic bacteria represent more than 50% of the total microbiota, with restrictions on the presence of butyric bacteria, coliform bacteria, and Pseudomonas genes [1, 5].

Simultaneously to avoid defects and the risk of transmitting pathogenic bacteria from diseased animals, after milk collection and its qualitative and quantitative verification, pasteurization in t/T regime that does not cause the denaturing of milk proteins and ensure destruction of transmissible pathogenic micro-organisms through milk. After cooling, the milk is inoculated with specific monocultures or mixed cultures, the bacteria are propagated simultaneously with the lactic fermentation, and the technological stages aim to direct the useful activity of the starter cultures [3,6].

It is considered that cheeses are not an ideal substrate for the development of all microorganisms, but some microorganisms, including selectioned microorganisms used as starter cultures in the cheese industry, are adapted to this type of biotope. Thus, after inoculation into pasteurized milk and cooled to the optimum temperature for cultures, bacteria produce lactic acid lactose fermentation and flavoring substances. For soft cheese varieties, lactic fermentation takes 4-6 hours until the pH of lactic acid decreases below 4.5 when acidic coagulation of milk casein occurs. For assortments of hard paste cheeses, for example, when the pH reaches 5, in the fermented milk is added proteolytic enzymes from the clot, active to this pH, and enzymatic coagulation occurs [2,4].

Therefore, in milk is performed the multiplication of lactic acid bacteria favored by the presence of lactose, by assimilable nitrogen sources (amino acids and peptides), by favorable oxidation potential. The main role of lactic bacteria in cheese making is the intense and safe production of lactic acid [7,8,9].

Studying various bibliographic literature of different authors, I found it appropriate to carry out some scientific researches in this field and for this reason I proposed the main objective of this study to be the study of microbiological aspects on the importance of bacterial cultures used in the production of the important milk product - cheeses.

Material and method

For performing the research, were conducted bacterioscopic and bacteriological investigations of homemade cheeses.

Microbiological investigations were performed based on the samples of cow, sheep and goat cheeses according to the microbiological laboratory pattern.

Results and discussions

The detailed analysis of the researches made it possible to detect and analyze the microbiological aspects based on the detection of the number of lactic microorganisms through the lactic bacterial species (Streptococcus thermophillus, Lactococcus, Lactobacillus etc.) studied by their cultural activity, mode of action and other features that are particularly complex and important.

According to the researches on dairy products containing microbial lactic species, these represent a particular interest and manifest their activity through a variety of beneficial effects on the organism (Table 1).

Table 1. Cultures of lactic bacteria used as production cultures for cheese production

Cultures of microorganisms	Types of cheeses
Lactococcus lactis Lactococcus lactis diacetilactis	Fresh cow cheese
Lactococcus lactis Lactobacillus casei	Telemea, curd for consumption
Lactococcus lactis Lactococcus lactis sp. Cremoris Lactobacillus casei	White cheeses in brine Mingled cheeses
Lactococcus lactis Streptococcus thermophillus Lactobacillus casei	Scalding cheeses
Lactococcus lactis Lactococcus lactis sp. Cremoris Lactococcus lactis diacetilactis	Semi hard cheeses Netherlands

Species of lactic microorganisms are of particular importance to lactic products by some determinations of interactions between lactic bacteria that are particularly complex and beneficial to stimulate the growth of fermentative activities. Thus, S. thermophilus does not possess sufficient extracellular proteolytic activity, and the amount of free amino acids and peptides in milk is not sufficient for its optimal growth (Table 2).

Table 2. Microbial species present in researched cheeses

Cheeses	Microbial species
Cow	Lactococcus cremoris, Streptococcus lactis
Sheep	Lactobacillus bulgaricus, Streptococcus lactis
Goat	Lactobacillus plantarum, Lactococcus cremoris

It is important to avoid defects and the risk of transmitting pathogenic bacteria from diseased animals, after milk collection and its qualitative and quantitative verification is carried out

in t/T pasteurization that does not produce milk proteins and ensure destruction of transmissible pathogenic micro-organisms through milk. After cooling, the milk is inoculated with specific monocultures or mixed cultures, the bacteria are multiplied simultaneously with the lactic fermentation, and the technological stages aim to direct the useful activity of the starter cultures (Table 3).

Thus, coagulation of milk is accompanied by the conversion of soluble casein in insoluble paracasein which is performed in two ways: coagulation of acid may take place due to the accumulation of lactic acid resulting from the fermentation when the lowering of the pH reaches the isoelectric point of casein from milk; insoluble paracaseinate and calcium lactate are formed; enzymatic coagulation is performed with coagulant proteolytic enzymes when a compact, richer calcium coagulum (Figure 1) is obtained by insoluble paracaseinate formation.

Table 3. Conditions for reactivation the lactic bacteria cultures

Milk product	**Selectioned cultures**	**Inoculated quantity, %**	**Temperature, °C**	**Time, hours**	**Acidity, °T**
Sour milk	Str. lactis, cremoris, lactis (diacetilactis)	1-2 1-2	20-23 25-30	17-20 18-24	90-95 86-92
Fermentated sour cream	Str. Cremoris Str. acetoinicus	3-5	24	24	65
Yogurt	Lb. delbruechi (bulgaricus) Str. salivarus (thermophilus)	1-2	43-45	3	90-95
Acidophilic milk	Lb. acidophillus	2-3	37-40	7-10	120-130
Fresh cheese	Streptococcus	2-3	26-30	12-24	100

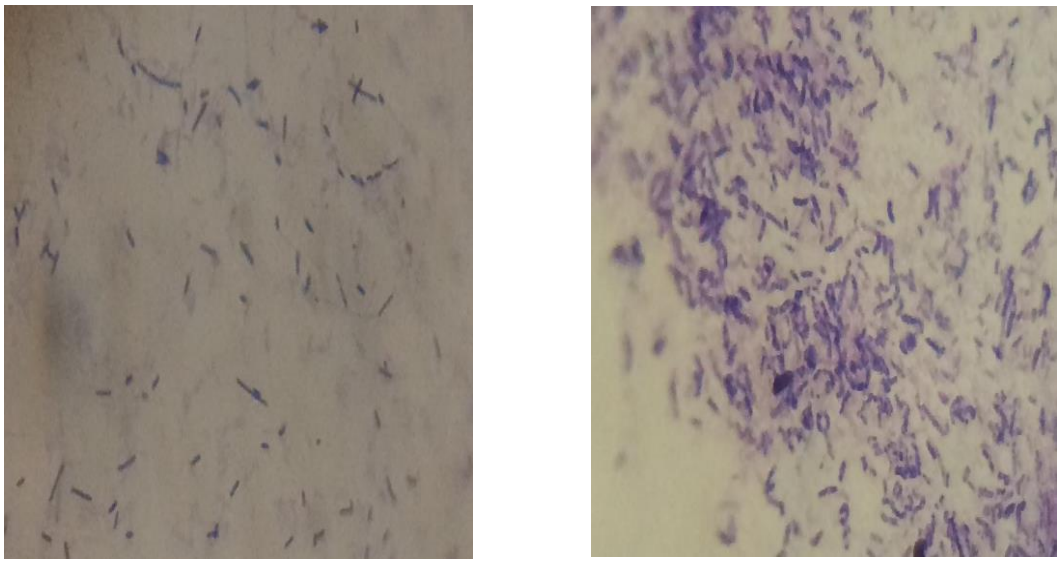

Fig.1. Lactic microorganisms

Nowadays, the coagulating enzymes are used: the curd, obtained by extraction from lamb stomach (calf), containing a complex of proteolytic enzymes which includes: chymotrypsin, trypsin, plasmin renin; enzymes of plant origin: papain, bromelain, ficin, used in warm countries; enzymes of microbial nature are obtained with the help of microorganisms selected with the following species: *Mucor pussilus, Mucor miehei, Endothia parasitica*.

The coagulation process is performed according to the assortment of cheese, the coagul is subjected to the cutting operation (grinding), which favours the separation of the whey. During the operation, bacteria are multiplied and by separating the whey it is estimated that about two-thirds of the milk microbe remains in the clot and the rest is eliminated with the whey.

The maturation of cheeses is a very important step for the finished product and is the result of complex activity of microbial live cells, of microbial exogenous enzymes or of released enzymes from the process of autolysis of nonviable cells and of native milk enzymes or added with the clot on the main components of the clot, namely: unfermented lactose, lactic acid, antide and lipids.

As a result of the formation of calcium lactate, the acidity of the paste is reduced and the activity of the bacteria which requires the development of neutral pH values is possible. At maturation, a limited hydrolysis of milk proteins, especially casein, occurs with the formation of low molecular weight compounds, readily digestible and amino acids. By maturation, the digestibility and nutritional value increase. Amino acids resulting from maturation can undergo transamination, decarboxylation, deamination, and the resulting products can be aldehydes, alcohols, etc., which explains the diversity of taste and aroma.

Lipids under the action of lipases produced by cultures or from clot or milk are converted to glycerol, a source of carbon for microorganisms, and fatty acids are partially converted into aldehydes, ketones, compounds that give a spicy, specific taste.

Taking into account the subsequent information, we note the importance of microbial digestion of acidic dairy products characterized by nutritional value and high biological value, because by the consumption of lactic bacteria (occasionally yeasts), man benefits from the presence of vitamins produced by these microorganisms. Lactic acid bacteria can produce antibiotic-like substances such as: bulgaric acid, acidophilin, which may speed up the healing of gastrointestinal diseases. Some lactic acid bacteria can adapt to the human body because they have an optimal temperature of 37ºC, such as those isolated from infants intestinal microbiota (Lactobacillus acidophillus and Lactobacillus bifidus).

Considering the cheese making technologies, we are finalizing this study as a basis for the use of selectioned lactic bacteria cultures, which are used in the form of monocultures or mixed cultures with the appropriate biotechnological properties for obtaining quality products in everyday life of the man.

Conclusions

1. The lactic bacteria in cheeses favor the presence of lactose, of assimilable sources of nitrogen (amino acids and peptides), of favorable oxidation potential
2. The principal role of lactic bacteria in cheese producing is the intense and safe production of lactic acid.
3. Nowadays, from the coagulant enzyme is used the clot obtained by extraction from the lamb stomach (calf), which contains a complex of proteolytic enzymes.
4. The maturation of cheeses is a very important step for the quality of the finished product and is the result of the complex activity of microbial live cells, of microbial enzymes.
5. The isolation of useful microbial cultures regarding the microbial biotechnology of cheeses characterizes the nutritional value and high biological value of this dairy product food.

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The immunological aspects regarding the importance of the phagocytic indices at ovines

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Abstract
The scientific investigations revealed in this research had the purpose to study some immunological aspects regarding the importance of the phagocytic indices at ovines in different physiological periods and at lambs in different periods of age. The obtained data, reflects that the cellular defense mechanisms against bacteria are performed by lymphocyte cells equipped with phagocytic macrophage functions, neutrophils, etc. This determines the importance of the phagocytic mechanisms in triggering and controlling cellular reactions. The results of the research reveal the evaluation of the cell phagocytic indices at ovines and lambs by establishing the significant values of both the phagocytic activity and the phagocytic intensity. These researches give us the possibility to conclude that the installation of the immunological reactivity of the animal body and the adaptation to changes in the environment, especially the action of the pathogens, take place.
Key words: *Immunity, Ovines, Lambs, Phagocytic Activity, Phagocytic Intensity.*

Introduction

The immune function of the human and animal organism has not only the classic role of antiinfectious defence, but it is also in a continuous expansion of its implication, both in maintaining the chemical homeostasis of the body and in numerous pathological states. The immune system is essential for the survival of multicellular organisms, due to the permanent aggression of infectious agents. Human beings and animals carry a huge number of bacterial cells (about 10^{14}) on their mucous membranes and skin, more than their own cells, some of which have the potential to initiate infectious processes [7; 8; 4].

The concept of cell mediated immunity shows that there are a number of specialized cells in the body with the ability to recognize and enclose foreign cells through the phagocytosis process, which are digested and removed from the phagocytic cell. At the same time, observations have been made on the importance phagocytic cells that contain and digest fungal spores, determining the aspects of spore infections and revealing the body's ability to defend [1; 3; 5].

Those two doctrines of the immune phenomenon, which have been confronted by their representatives, have been unified and demonstrate the existence in the serum of natural antibodies called opsonin, which act in co-operation with phagocytes. They have a particular important implication in the protection of the newborn organism against infections, intervening in defense processes by phagocytosis and pinocytosis, followed by the release of lithic enzymes, causing the destruction of microorganisms [2; 6; 9].

Bibliographical studies confirms that humans and animals produce one billion daily passing circulating lymphocytes. By circulating and recirculating through the blood and lymphatic vessels network, immune cells and molecules provide body surveillance, recognition of nonself molecules and cells to eliminate them [9; 11].

The study of immune system cells reflects their cooperation in reciprocal formation of reaction needs, which are mutually controlled in their activity by lymphocyte or macrophage secreted molecules, with an extremely important role in the organization and control of immune reactions [10; 12] . From this point of view, the *main objectives of these researches are the study*

of the activity of cellular immunity determined by phagocytic indices at ovines and lambs at different age periods

Material and method

The investigations were performed in the laboratory of microbiology and immunology of the Clinical-2 Department from the Faculty of Veterinary Medicine of the State Agrarian University of Moldova. To perform the investigations were used blood samples from ovines and lambs from a household of a sheep farm.

Blood samples were collected from the heparin jugular vein based on the calculation of 0.3 ml of heparin per 10 ml of blood for anticoagulation. Samples were used to identify opsono-phagocytic indices.

For the opsono-phagocytic test, 1.0 ml of heparin-stabilized blood and 0.1 ml of E. coli microbial culture suspension were used from 1.0 ml physiological solution at 500 ml. microbial cells. The tubes were subjected to agitation, then incubated in the thermostat for 30 minutes at T-37 C and centrifuged at 1500 rpm. The supernatant was removed via the Pasteur pipette. Colored smears were performed using the Romanovschii-Giemsa method, fixed with methyl alcohol and colored over 30 minutes.

Preparations from the blood samples were visualized under a 90-well immersion microscope. The test determined the number of phagocytic microorganisms per 100 neutrophils. The index of activity and phagocytic intensity was determined by determining the percentage of neutrophil cells involved in the phagocytosis process. In the same time phagocytic intensity was determined by the number of micro-organisms embedded in a single neutrophil. The calculation was made by expressing the ratio of the sum of the phagocyte microorganisms to the number of neutrophils involved in the reaction.

Results and discussions

The results of immunological investigations on the immune system regarding phagocytic indexes at ovines and lambs show that the level of phagocytic indices vary at different stages of their physiological states.

Significant results of the phagocytic activity indices at ovines before the gestation and postpartum periods (Table 1) determined values equal to 54.45 ± 1.22 and 51.40 ± 1.72 compared to phagocytic intensity values, where the indices constituted the level of 5.04 ± 0.13 and 5.00 ± 0.11.

Table 1. The dynamics of phagocytic indices at ovines during different physiological states

Ovines	**n**	**Phagocytic activity**	**Phagocytic intensity**
Ovines before gestation	5	54,45 ± 1,22	5,04 ± 0,13
Postpartum ovines	5	51,40 ± 1,72	5,00 ± 0,11

Therefore, the animal organism as a functional system is balanced as long as the antigenic information it receives is identical to its own. With respect to foreign molecules, which deviate from their own information model, the immune system responds by activating recognition mechanisms to remove nonself molecules. Therefore, the assembly of complex cascade phenomena triggered by the specific interaction of the immune system with the antigen, during which the lymphocyte cells activate, proliferates and constitutes the immune response.

Thus, the mechanism of immune response regulation is based on the phagocytic immune response controlled by regulatory systems of at least equal complexity with those underlying their triggering and expression. In this context, in the case of blocking regulatory mechanisms, clonal proliferation or immunoglobulin synthesis can no longer be limited, resulting in the profound alteration of the immune response, accompanied by the establishment and evolution of diseases that usually have a fatal outcome.

These aspects indicate that in addition to these processes, cellular defense mechanisms against bacteria are performed by effector cells with phagocytic functions (macrophages, neutrophils, etc.). Through various cellular mediated mechanisms, macrophages undergo activation by secreted lymphokines by some types of lymphocytes. They denote the importance of phagocytic mechanisms in triggering and controlling cellular responses.

The study of immune defense mechanisms at lambs revealed various indices characteristic of phagocytic activity and intensity varying at different age groups. Thus, phagocytic activity indices determined significant values at various age ranges (Table 2). These data indicate that animals have resistance to infectious germs.

The dynamics of these indices demonstrates that at lambs aged 10 days the phagocytic activity constituted 29.4 ± 0.31 compared to the age of 20 days, which constituted 37.5 ± 0.42, which indicates a decrease expressed by various aspects of external factors that act on new-born lambs at this age. Following the dynamics of the phagocytic activity indices at the age of 20 and 30 days it was found that the values constituted 37.5 ± 0.42 and 40.6 ± 0.45, which confirms the diminution of the phagocytic processes at these animals.

Table 2. The dynamics of the phagocytic indices at lambs depending on age

Age (days)	**n**	**Phagocytic activity**	**Phagocytic intensity**
10	5	29,4 ± 0,31	1,86 ± 0,03
20	5	37,5 ± 0,42	1,62 ± 0,12
30	5	40,6 ± 0,45	2,81 ± 0,04

In the immunological aspect, it can be observed that the phagocytic activity during this period of lambs life is attributed to the neutrophils, the rest being done by macrophages. Therefore, phagocytic mechanisms induce two-way phenomena that are dependent on bacterial resistance: the first pathway without opsonization through direct interaction between the phagocytic cell and the antigen; and the second pathway with opsonization is the interaction that requires an additional opsonin molecule that acts as an adapter between bacteria and leukocyte.

Relevant data were registered regarding the phagocytic intensity of lambs investigated at different age periods reported in Table 2. From the results we show that the phagocytic intensity at lambs aged 10 days determined significant values of 1.86 ± 0.03, compared to animals aged 20 and 30 days, where these values were 1.62 ± 0.12 and 2.81 ± 0.04. Therefore, at neonatal animals, defense mechanisms are not triggered enough to protect the aggression of microorganisms, viruses and other pathogens.

The development of immunity or tolerance is subject to fine-tuning mechanisms because the immune response to self-antigens or the tolerance of a pathogenic potential may have unfavorable consequences for life. The regulation of humoral or cellular immune response is a complex modulation process in which there are a number of means by which specific body defense is maintained at a certain level and with a certain duration in order to achieve homeostasis and preserve health.

Based on these considerations, the evaluation of phagocytic cell indices at ovines and lambs registered significant values of both phagocytic activity and phagocytic intensity. These findings allow us to conclude that immunological reactivity of the newborn organism is occurring and adaptation to changes in environmental conditions and especially to the action of pathogens. In addition to these processes, cellular defense mechanisms against bacteria are performed by effector cells with phagocytic functions (macrophages, neutrophils, etc.). Through various cell-mediated mechanisms, macrophages undergo activation by lymphokines secreted by T lymphocytes. They denote the importance of phagocytes and macrophages in triggering and controlling cellular responses.

Conclusions

The evaluation of the mechanisms of immune system formation of the ovine animal organism provides the opportunity to follow the evolution of cellular and humoral responses, which maintain immune homeostasis of the organism, cellular and humoral protective factors considered as key to the regulation of the immune system.

The results, regarding phagocytic activity during this period of lambs life, are attributed to neutrophils, the rest being done by macrophages.

The values of the phagocytic intensity of the investigated lambs at different age periods determined significant values, which confirm that the defense mechanisms are not triggered enough to protect the aggression of microorganisms, viruses and other pathogens.

The performed study on immunological investigations on phagocytic activity at ovines and lambs determined significant important values at the appearance and development of the cellular immunity.

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The assesment of domestic cats body condition through direct and indirect methods

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Abstract
The cats inability to adapt their enzymatic package to a domestic lifestyle and a restricted space of movement , for cats raised in appartments , made them more prone to overweight and obesity. Body scoring is a method of evaluation subjective and semiquantitative through inspection and palpation. Through the inspection and palpation of the fat that covers the chest , the presence or absence of the abdominal waist and the size of the belly fat are enough to establish the body score for cats. The biochemistry results showed significantlu changes of the parameters leptin, insulin, plasma total ghrelin, cholesterol and tryglicerides, which sustains their role in the adaptation of cats to a positive or negative energy balance. The purpose of this study is to change the owners perspective regarding the way they treat the physiologycal needs of their pets. They ignore their cats need for space, unlimited acces outside, their needs regarding the food, especially the increased requirements of proteins. The owners tend to mistakenly believe that a cat with an ideal score is underweight and that overweight cats are an ideal score.
Keywords: *cats, body score, overweight, leptin, plasma total ghrelin.*

Introduction

Metabolic disorders are caused by the increased need for a particular element or nutrient, which becomes deficient under certain conditions. Metabolic storage disorders are the result of the body's inability to break down certain substances into the partial or total lack of the necessary enzymes. Substances can accumulate in the body to toxic levels or the body cannot produce a certain substance it needs. Clinical signs appear within weeks or months after birth. These diseases are usually fatal (Zoran Debra, 2002). Emaciation, marked weakening, pathological hypotrophy of tissues, are manifestations of deficiencies of supply, parasitism, tumor and infectious diseases. The terms "overweight" and "obese" are used to denote the gravity of excessive fat mass. Individuals are classified as overweight if they exceed 10% to 20% of their ideal body weight and as obese when they exceed more than 20% of their ideal body weight (Loyd C., 2014). There are more complex assessment systems, such as body score that combine visual assessment and palpation of adipose tissue mass to divide body composition into several predefined categories. A number of metabolic changes occur, which may not be evident through a routine laboratory evaluation. Therefore, it is very important for clinicians and owners, periodic monitoring of weight changes and to initiate appropriate actions to prevent or treat unwanted weight gain (Russell et al, 2000).

Materials and methods

The purpose of the present study was to establish the body score of the cats included in the study and the effects of weight gain, of the overweight on the plasma concentration level of the hormones ghrelin, leptin and insulin.

In the study entered 20 cats: 11 females, of which 7 were sterilized and 4 were unsterilized and 9 were males, of which 7 were sterilized and 2 were intact. This study was performed by morphometric analysis, respectively body score with 5-point reference scale, biochemical analysis, hematological examination and determination of hormones ghrelin, leptin and insulin, as well as

by coproparasitological examination. The study was conducted in the period 2018-2019 in collaboration with the cat owners.

The history of the evaluated cats followed the situation of deworming and vaccination, breeding environment, gender, breed, age and a brief description of the nutritional history. The 20 cats did not show any clinical or subclinical signs of major pathologies before or after evaluation.

In the diagnosis of cats an important weight had the visual physical evaluation and by palpation, respectively the body score and the determination of the body weight by weighing. Body score is a method of subjective and semi-quantitative physical evaluation.

Blood samples were collected from the jugular vein to assess glucose, cholesterol, triglyceride, total lipid, total protein, PAL, urea and leptin, ghrelin and insulin levels. These parameters were determined using a UV Vis Screen Master Touch spectrophotometer, based on the End Point colorimetric reaction, after incubation at 37 ° C, according to the time indicated on the package leaflet. The plasma concentration of ghrelin and leptin was determined by ELISA, and the concentration of insulin by an immunochemical test with electrochemiluminescence detection, ECLA. Fecal samples were collected from the two underweight cats. To detect the presence of eggs and oocysts, the Willis flotation method was used. The principle of the method is based on the floating and lifting of light eggs and oocysts on the surface of saturated sodium chloride solution and their adhesion on the glass blade.

Results and discussions

Of the 20 cats examined, following the morphometric evaluation, it was established that two cats are underweight, one unsterilized female and one unsterilized male, 12 have an ideal body score, of which 4 are sterilized males, 4 sterilized females, 2 intact males and 3 intact females, and 6 cats are overweight, of which 2 are sterilized males, 3 non-sterilized females and one intact female. From the case study included, 3 are fed only on dry food, and 17 receive both wet and dry food, the overweight cats being included in the category of those receiving both wet and dry food.

After examining the smears of the 20 cats classified in the case, we observed in the 6 overweight cats, changes in the form of red blood cells, poikilocytosis, red blood cells with irregular margins. This change in shape occurs due to the oxidative stress that occurs as a consequence of the presence of excess adipose tissue. Other changes in leukocytes or platelets were not observed.

The biochemical examination of the 6 overweight cats revealed changes in cholesterol, ranging from 260 mg / dl to 401 mg / dl and triglycerides, with values ranging from 83.1 mg / dl to 189 mg / dl. Following the determinations of the plasma concentrations of the hormones in the 6 cases of overweight, we found the decrease of the plasma concentration of ghrelin in all 6 patients, together with the increase in the concentration of leptin and insulin. The values of the ghrelin varied between 2020-2320 pg / ml, much lower compared to the normal values of 4000 pg / ml, the values of leptin ranged from 8.9 to 9.9 Ng / ml, well above the normal values of 2 Ng / ml and insulin values, 76-91 Pmol / l, increased above normal, 60 Pmol / l.

Following the macroscopic examination of the faeces collected from patient number 13, the presence of ovular capsules was observed, and following the determinations made by the Willis flotation method, we showed under the microscope, the presence of cestode oncospheres and the diagnosis of digestive cestodosis was made. In case number 9, the presence of the eggs of *Toxocara cati* was found and the diagnosis of toxocariasis was made.

It has been demonstrated the hypothesis that in the case of overweight cats, as long as the leptin concentrations are higher, the insulin resistance of the cat will increase. Apleton discovered in 2002 that, the increase in the percentage of body fat in cats is accompanied by increased insulin

resistance, respectively, increased insulin compensatory secretion and increased leptin concentration (Appleton et al, 2002).

Laurence Colliard's 2008 study showed that the owners, from a visual point of view, underestimate the body score of cats, considering cats with ideal score, normal weight as underweight, which makes the owners to feed them more, thus increasing the chances that over time cats become overweight or even obese (Colliard et al, 2008).

Conclusions

Of the 20 cases examined by visual inspection, palpation and weighing, 6 cats are overweight, of which 2 are male and 4 are female, and 2 cats are underweight, one female and one male unsterilized. Of the 6 overweight cats, 5 are sterilized and one unsterilized female, which shows the high incidence of weight gain in sterilized cats. None of the patients examined were found to have diabetes, blood glucose levels were within normal limits. The 2 underweight cats were positive at the co-parasitological examination, being infested with nematode eggs and cestode oncospheres. Hematological determinations, namely examination of blood film, revealed in the overweight patients, changes the shape of the erythrocytes, the irregular margins. This change of shape occurs against the background of oxidative stress that the body is subjected due to the presence of excess body fat.

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Macroscopic anatomical features of the digestive system in the common buzzard (*Buteo Buteo)*

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Abstract
The study was conducted on 10 bodies of common buzzard (Buteo buteo), from the Association for the Protection of Birds and Nature, MILVUS Group. The bodies were processed according to the dissection protocol established to highlight the macroscopic anatomical features, characteristic of the order in which the studied birds belong. The macroscopic aspect of the esophagus, which is short, distensable, has a well-developed crop, located cranially in the thoraco-abdominal cavity. The glandular stomach and the muscular stomach are poorly developed, joining into the thoracic-abdominal cavity, giving a pear-like appearance. The small intestine is short, divided into the duodenum, jejunum and ileum, and the large intestine has a vestigial, lymphoid type caeca and a short colon. The liver composedof two lobes that join cranially in midline. The right lobe is larger than the left lobe. A well developed gallbladder is located on the ventral side of the right lobe.
Keywords: *Common buzzard, groin, glandular stomach, muscular stomach, ceaca*

Introduction

The morphology of the gastrointestinal tract, metabolic capacity and the physiology of digestion intersected during evolution to match the nutritional needs, depending on the foods available in the natural habitat. (Kirk C. Klasing, 1999) The digestive system is also the largest immunological organ in the body, protecting the bird against exogenous pathogens. (D. Michael Denbow, 2000)

The aim of this study was to supplement the general existing notions, regarding the morphology of the digestive system of the common buzzard (Buteo buteo), highlighting the particularities and how the specific and strictly carnivorous diet, has modified the digestive system in this species. The Common Buzzard (Buteo buteo) is a common bird in our country, being found in both wooded areas and extensive plains.

Materials and methods

The dissections were performed on 10 bird carcasses, all belonging to the family Accipritridae, the genus Buteo, the species Buteo buteo, the Common Buzzard, in the Faculty of Veterinary Medicine, the discipline of Comparative Anatomy.

The birds were brought from the Târgu Mureș, through the kindness of our colleagues from MILVUS Group, Bird and Nature Protection Association. They are birds protected by law (law 49/2007, law 04/2011, law 51/2011 etc.), so there are restrictions regarding interaction with them, but by Directive 2009/147 / EC of the European Parliament and of the Council of November 30, 2009 on the conservation of wild birds, Article 9, point 1 (b), they can be used for research purposes. The birds brought died after accidents (electrocuted, road accident), or were euthanized due to injuries that no longer allowed them to be rehabilitated in the wild.

The body was opened in several stages. The protocol was developed following some passages from the book of Veterinary Necropsic Diagnosis, Cornel Cătoi, 2003, AcademicPres Publishing House.

The plucking of the corpses was carried out strictly in the incision area. At the level of the head, the skin is incised from the level of the lateral commissure of the beak. The incision of the skin continues on the ventral side of the neck, lateral to trachea, up to the level of the cloacal orifice. With scissors, the abdominal muscles are transversely sectioned, at the posterior part of the sternum, posterior to the xiphoid appendix. On each side of the sternum, the initial abdominal incision continues, up to the level of the chondrosteal junctions. The abdominal wall is sectioned longitudinally, up to the bladder and broken laterally. Continue the sectioning with scissors of the chondrostal joints, bilaterally, up to the level of the scapulohumeral joints, the coracoid bones and the clavicle is cut, the sternum is removed, after the pericardial sac is disintegrated. The organs located in the cavity are detached, both commissures of the beak were cut, with the lower jaw detached, together with the esophagus and a portion of the trachea. The skin and the musculature adjacent to the cloacal orifice are cut in order to detach it, together with the digestive tract and the attached organs.

The digestive tract was examined only macroscopically, in situ, and separately from the carcass. It opened, with a scissors, the esophagus, the glandular stomach, the shredding stomach, the small intestine, the large intestine, the cloacal orifice.

A digital camera, Nikon COOLPIX P900, was used to produce the images.

Results and discussions

The beak is strong and curved, includes parts of the upper and lower jaws, the upper jaws are better developed compared to the lower jaws, being covered on the outside by several hard keratin patches, or rhamphotheca. The rhamphotheca is divided in its turn into the rhinotheca, the sheath that covers the maxillary portion, and the gnathotheca, the sheath that covers the jaw. The maxillary rhamphotheca presents at the base a fleshy formation, called ceroma, which represents the boundary between the beak and the frontal part of the head, a formation that also includes the two nostrils. The medial dorsal border of the maxillary rhamphotheca is called culmen, and the ventral median border of the mandibular rhamphotheca is called the gonys.(B. Speer, 2016) The sharp edge of the frame is called the tomia. Birds lack a soft palate and a pharybgeal isthmus; the combined oral and pharyngeal cavities are referred to as the oropharynx.(D. Dendow, 2000, C. Lacasse, 2015) On the hard palace are the choana, longitudinal fissure, which connects the oral and nasal cavities. Infundibular fissure is a common pathway for the ear canal. Palatal ridges, tongue, glottal process show epithelial projections, cornified. The tongue is slightly mobile, on the dorsal surface the papillae are organized in the form of the letter V, with an opening in the aboral sense. (K. Klasing, 1999)(Figure 1)

The laryngeal protuberance presents on the rostral surface the glottis, which represents the entrance to the larynx. Esophagus has two portions: the cervical portion, which is short, and the thoracic portion, which is longer. The crop represents an expansion of the esophagus, present in all raptors, except owls. The raptos crop is fusiform-shaped and has a poorly developed lower sphincter into the thoracic esophagus.(S.Ford, 2010) The esophagus is continued with the proventriculum or the glandular stomach, without a clear demarcation. (Figure 2).

Fig.1 Jaw: 1. Tongue; 2. Laryngeal protuberance with glottal orifice; 3.The esophageal orifice
Maxilla: 4. Choana; 5. Infundibular fissure
The presence of cornified papillae is indicated with the help of arrows

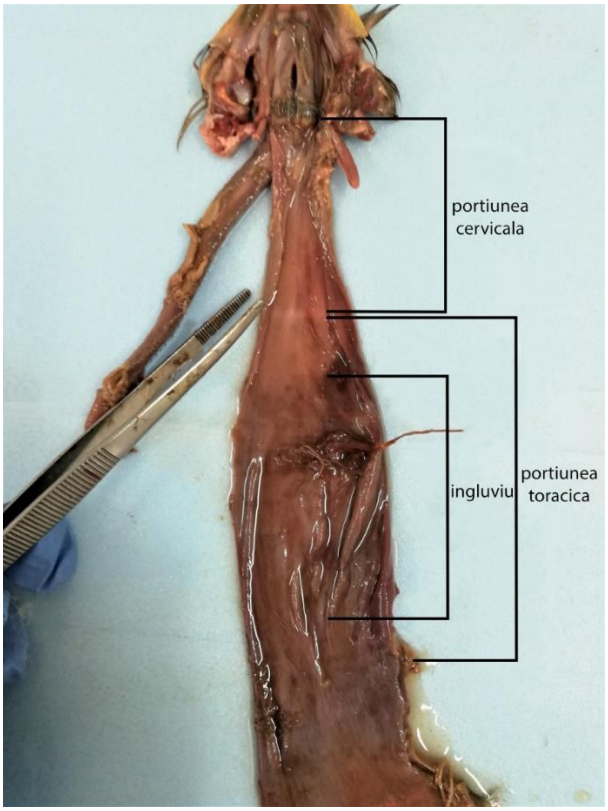

Fig 2. Delimitation of the esophagus and highlighting the groin,
located in the last esophageal segment

The proventriculus or glandular stomach is relatively small in size and is interconnected to the thin wall of the ventriculus or the muscular stomach. (D. Denbow, 2000) The intermediate area between the proventriculus (glandular stomach) and ventriculus (muscular stomach) is dominated by an isthmus.(Figure 3) In addition, the proventriculus and ventriculus form one large pear-shaped cavity. (S.Ford, 2010) Intestinal tract is located in the caudal portion of the thoraco-abdominal cavity, being a compact mass, surrounded by adipose tissue. The posterior portion of the ventricle narrows toward the pyloric region, located on the right side of the organ, 90 ° from the longitudinal axis of the proventricle.(N.Barton,D.Houston,1993) Small intestine is shorter, extending from the level of the pyloric region of the ventricle to the level of the cecum and colon. Duodenal portion is relatively long; there is no obvious crossing between the duodenum and ileum. The demarcation is done according to the anatomical position (M. Murray, 2014, E. Klaphake, J. Clancy, 2005).

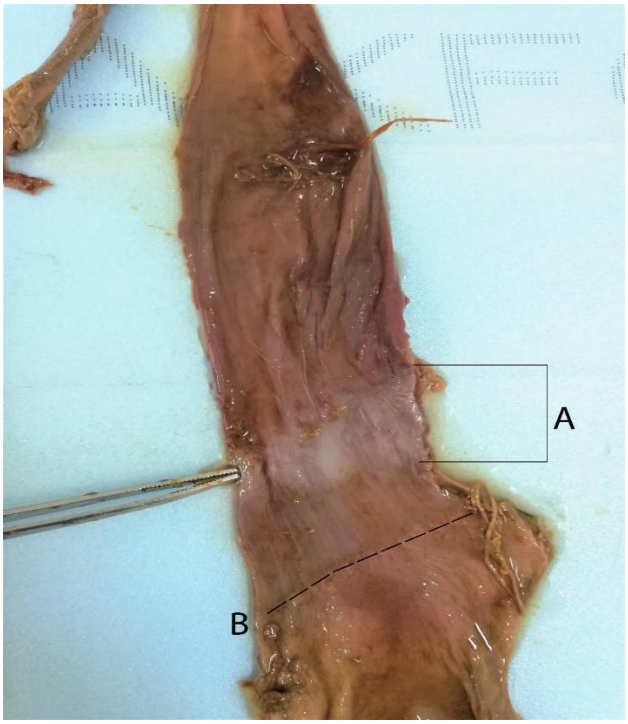

Fig.3 The passage between the glandular or proventric stomach (A) and the shredding or ventricular stomach (B) is dominated by an isthmus (indicated by an arrow).

Colon (sometimes called the rectum) is short, extending from the ileo-cecal junction to the cloaca. Cecum is represented by two rudimentary, vestigial formations. (K.Klasing, 1999) (Figure 4) The birds of the order Accipitriformes, the order that the studied birds belong (the Common Buzzard), are somewhat lymphoid. (Scot Ford, 2010) The cloaca extends from the distal portion of the rectum to the anal orifice.

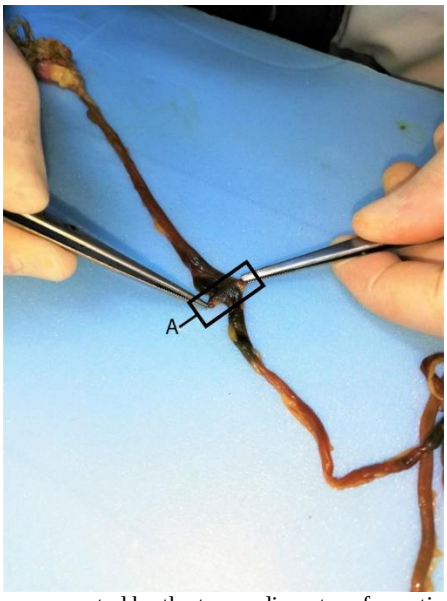

Fig 4.(A) Cecum, represented by the two rudimentary formations, characteristic for the birds of the Accipitriform order

Organs attached to the digestive tract are the liver and pancreas. Pancreas could not be highlighted due to autolysis, the bodies being opened after 3 weeks. Liver is composed of two lobes, which unite cranially, along the midline, extend to the thoracic-andominal cavity and surround the apex of the heart. The right lobe is larger compared to the left lobe. The gallbladder, located on the ventral side of the right lobe, is well developed. (J.Samour, J.Naldo,2007)(Figure 5).

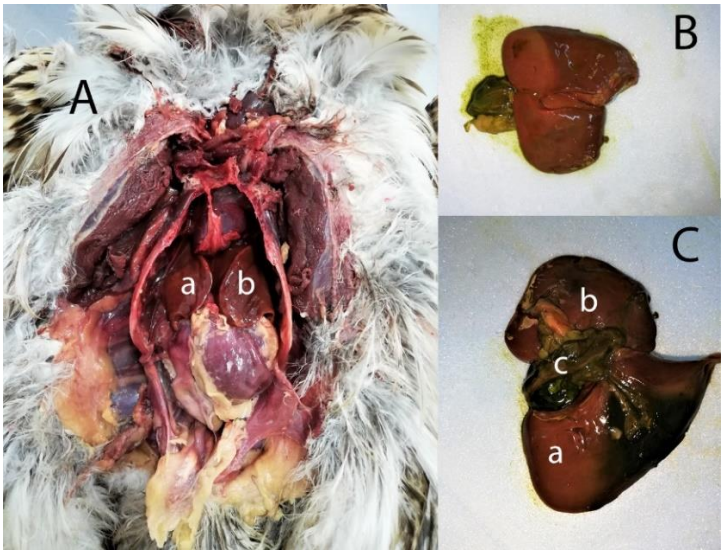

Fig. 5 (A) Liver in the thoracic-abdominal cavity. The two lobes, the right lobe (a) and the left lobe (b) join cranially to the midline, (B) The parietal face of the liver, with the two lobes, the right lobe and the left lobe and the gallbladder, (C) The visceral face of of the liver, with the two lobes, the right lobe (a), visibly better developed in comparison with the left lobe (b), and the gallbladder

Conclusions

1. The digestive system is adapted to the type of food, strictly carnivorous. Birds eating high-protein diets generally have less complicated digestive systems than those eating complex carbohydrates.
2. The beak is strong, curved and jaw muscle well developed, adapted for tearing prey, it was intact when we analysed all corpses. The tongue is less mobile and we could highlight the cornfield papillae on its surface. The soft palate is absent, presenting a single cavity – oropharyngeal cavity.
3. We located the esophagus on the right side of the neck. It can expand in diameter due to the longitudinal fold. The presence of the crop, cranially located in the thoracic cavity, is designed to store ingested food.
4. The glandular and the muscular stomach are underdeveloped in comparison with the non-carnivorous birds, having a pear-like appearance.
5. The small intestine is reduced in size and there is no obvious macroscopic transition between duodenum and ileum.
6. The caeca, located at the junction between ileum and colon, reduced in size, is vestigial.
7. The colon is reduced in size, expanding from the ileocolin junction.
8. The liver is well developed, the right lobe is more developed compared with the left lobe.
9. The gallbladder is well developed, located on the ventral side of the right lobe.

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CT evaluation of HU bone density of the vertebrae in dogs with spine compression

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Abstract
Bone mineral density (BMD) is defined as the mineral concentration in bone. BMD is directly related to bone strength and is a useful predictor of osteoporotic fracture; it is therefore used to diagnose and monitor osteoporosis in humans. The purpose of this study was to evaluate if there are changes in the adjacent vertebral body (cranial and caudal) consistency in case of disk protrusion or IVDD. The result show changes of the HU of the vertebral body of the vertebrae situated cranial and caudal of the protrusion site, but there is no statistical correlation between the disk protrusion or IVDD and those changes.
Keywords*: IVDD, bode density, dog, CT*

Introduction

The canine intervertebral disc (IVD) is a versatile structure and is responsible for the stability and flexibility of the spine. IVD degeneration is a common phenomenon in dogs and is characterized by the degradation of the extracellular matrix, mainly proteoglycans and collagen. The medical definition of degeneration is: "tissue change into a less active or less functional form", and true degeneration is defined by the actual chemical change of the tissue itself (1).

Once the degenerative process has begun to trigger a cascade of events, these may eventually lead to a structural failure of IVD and the clinical signs of the disease. Common conditions related to IVD degeneration in dogs include: degenerative lumbosacral stenosis (DLSS), cervical spondylomyelopathy (CSM) and Hansen type 1 and 2 hernia. IVD hernia is the most common cause of neurological deficits in dogs with a lifetime prevalence estimated at 2%. However, IVD degeneration is not synonymous with IVD disease. While IVDs that lead to clinical signs will inevitably degenerate, degenerate IVDs are common findings in dogs (2).

The purpose of the paper is to determine if the disc protrusion in the dog induces changes in the bone density at the vertebral level, by performing measurements at the vertebra where the protusion occurs and by comparison with the neighboring vertebrae.

Material and methods

The study was conducted on 12 dogs, between the ages of 2 and 10 years, from the breed Bichon, Teckel and French Bulldog, in number of 5, 6 and 1 respectively; of these 6 were male and 6 females. The study was conducted at the Faculty of Veterinary Medicine in Cluj-Napoca, within the Radiology discipline for a period of one year.

The reason for the consultation being represented by different neurological symptoms, these being in correlation with the localization of the hernia, the degree of the protrusion, the time elapsed from the appearance of the symptoms and until the moment of the consultation, the temperament of the patient.

If the hernia was located at the thoracolumbar level, only the pelvic limbs were affected, that is to say paraplegia, and if the hernia was located at the cervical level, the thoracic limbs were also affected, in this case the term tetraplegia was used.

Anesthesia. For the correct examination, in the case of a radiological examination or a CT scan, the animal must be in a fixed position and undergo general anesthesia. The protocol used in anesthesia: intramuscular premedication with acepromazine in a concentration of 0.1 mg / kg, glycopyrrolate 0.01 mg / kg, butorphanol 0.1 mg / kg; intravenous thiamylal induction 10 mg / kg, intubation and maintenance with isoflurane in a circular narcosis system.

The examination was performed by computerized tomography. The CT images were obtained using a CT scanner (SOMATOM SCOPE, SIEMENS HEALTCARE, FMV CLUJ-NAPOCA). After anesthesia the patients are positioned on the CT table in the dorso-ventral position. The scanning conditions were as follows: 130 KVP and 130 mA, thickness of sections of 1mm, range of sections of 1mm. The 3D Net Medical program was used to visualize the vertebrae, respectively the disc herniation and to measure the bone density, in Hounsfield Units (HU), at this level. After examining the spine in the patients who had disc protrusion, we measured the bone density for 3 vertebrae in each patient: the vertebra where we observed the protrusion, the anterior vertebra, and the posterior vertebra respectively. Using different options from the program, we made several measurements on each vertebra, using the function "circle" by which we measured with approximation the diameter of the vertebral canal, the function "polygon" by which after drawing several lines we measured with approximation the diameter of the vertebral body, the volume, the maximum and minimum density at this level, respectively seven more points by which we measured the density in these areas, three of them being located in the middle of the vertebral body, on a vertical line starting from the middle of the medullary canal, two on the lateral parts of the vertebral body, and two on one side and the other of the medullary canal (Figure 1). In total we have measured the HU of 36 vertebras.

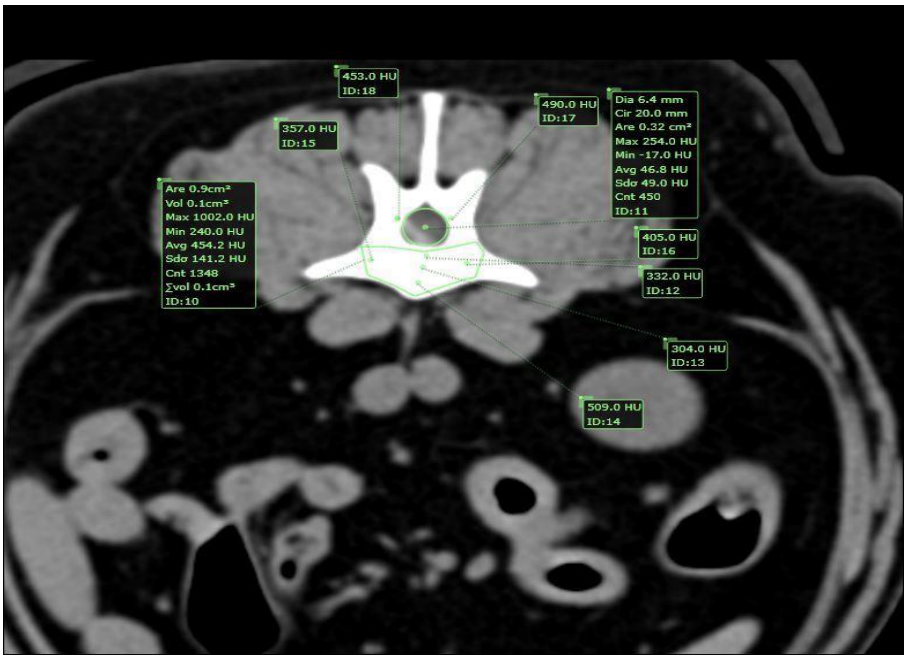

Fig. 1 Measuring the disk extrusion and the vertebral body density using Hounsfield Units

Results and discussion

Bone mineral density (BMD) is defined as the mineral concentration in bone. BMD is directly related to bone strength and is a useful predictor of osteoporotic fracture; it is therefore used to diagnose and monitor osteoporosis in humans. In human medicine, BMD is known to be affected by many factors, including age, sex, endocrine disease, gastrointestinal disease and certain medications. Osteoporosis or low BMD is a common condition that puts the patient at increased risk of pathological fracture; therefore, early diagnosis, prevention and monitoring of BMD are essential (2, 3).

Dual energy X-ray absorptiometry (DXA) is a standard, non-invasive and accurate method for measuring BMD and body composition in humans. Typically, central scans of DXA (lumbar vertebrae and proximal femur) are obtained, but DXA can be used to assess BMD throughout the body or at any specific location of the body. This method has several advantages, including cost-efficiency and fast scan time (3,4).

Quantitative computed tomography (QCT) is also used to measure BMD in humans and has a higher sensitivity than DXA, but a lower specificity for diagnosing osteoporosis. Although DXA is considered a standard BMD measurement technique, QCT is more sensitive than DXA for diagnosing osteoporosis and predicting the risk of pathological fracture, as trabecular BMD is lost faster than cortical BMD as the disease progresses. Decreased BMD in patients with metabolic or endocrine disorders is more evident in the trabecular bone than in the cortical bone, especially in the vertebrae. Measurement of trabecular BMD is essential for early detection of decreased bone mineral content, and QCT may be preferable compared to DXA (5,6).

Data obtained were recorded in a excel sheet and analyzed by using the paired two samples t-student test. Comparison of the media is performed to decide if there are significant differences between the studied environments, in our case whether the bone density changes or not in the vertebrae with protrusion compared to the neighboring ones.

We performed two analyzes, one between the caudal vertebra from the protrusion and the anterior vertebra and the other between the caudal vertebra from the protrusion and the posterior vertebra.

Most dogs with disc protrusion belonged to the Teckel and Bichon breeds, not observing a gender predisposition, the ratio being 50/50.

Depending on the area of the spine in which the protrusion occurred, 10 of the 12 cases concerned a vertebra in the lumbar region, and 2 cases in the thoracic region.

According to the topography of the disc, 3 of the protrusions were left ventro-lateral, 4 ventral and 5 ventro-lateral straight.

The diameter of the medullary canal narrowed between 5% and 50% in some cases due to protrusion.

The average of the 36 vertebrae measured at the vertebral body level was 515 HU (Hounsfield Unit) with a standard deviation of 91 HU, and the average of the same vertebrae, in which we measured 5 points at the vertebral body level and 2 points at the vertebral arch level, was 516 HU with a standard deviation of 74 HU.

Following the use of a t-test, in one of the 12 cases, a decrease in bone density was observed in comparison with the previous vertebra, but without statistical significance, the degeneration at this level could be the consequence of age, the dogs was 8 years, or due to metabolic deficiencies, genetics or other causes. The statistical significance was present for the t-student one tail sequence, a decrease in bone density being visible, base only on this case is hard to asses a correlation between disk protrusion and changes of the vertebral body HU values.

The HU values increase at the level of the vertebral canal where the protrusion occurs and the hyperattenuating disc material is present.

Following the measurements and analyzes, in some cases the HU values measured decrease at the level of the vertebrae where the disc protrusion occurs, without having statistical significance. Also, the increasing HU values of the vertebra where the lesion was identified were observed in 3 cases, without statistical significance. The decrease or increase in HU values cannot be correlated with the disc protrusion.

Conclusions

In conclusion, the disc protrusion itself does not cause a change in bone density at the level of the affected vertebrae. The lot in the study is to small and inhomogeneous, further study targeted on a specific breed and on a large number of individuals are required in order to establish a proper correlation between the disk protrusion of IVDD and changes in the bone density of the vertebral body.

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